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37 C.F.R. 1.8

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Date

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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Vladimir L. Makarov
John P. Langmore

Group Art Unit: 1631

Examiner: Unknown

Serial No.: 09/801,346

Filed: March 6, 2001

Atty. Dkt. No.: UMIC:039USC1/DLP

For: COMPOSITIONS AND METHODS FOR
ANALYSIS OF NUCLEIC ACIDS

RESPONSE TO NOTICE OF INCOMPLETE REPLY
DATED JULY 10, 2001

Commissioner for Patents
Washington, D.C. 20231

Commissioner:

In response to the Notice of Incomplete Reply mailed July 10, 2001, Applicants hereby provide substitute drawings in compliance with 37 CFR 1.84. These are the same drawing that have been accepted and published in the parent application, now U.S. Patent 6,197,557. A copy of the Notice of Incomplete Reply is also enclosed.

Pursuant to 37 C.F.R. § 1.136(a), for an extension of time of one month to and including August 10, 2001 in which to respond to the Notice of Incomplete Reply dated July 10, 2001.

Pursuant to 37 C.F.R. § 1.17, a check in the amount of \$55.00 is enclosed as the process fee for a one-month extension of time.

If the check is inadvertently omitted, or should any additional fees under 37 C.F.R. §§ 1.16 to 1.21 be required for any reason relating to the enclosed materials, or should an overpayment be included herein, the Commissioner is authorized to deduct or credit said fees from or to Fulbright & Jaworski L.L.P. Account No.: 50-1212/10101172/DP01982.

Respectfully submitted,

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Reg. No. 32,165
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Date: August 3, 2001

20010803 14:04:00

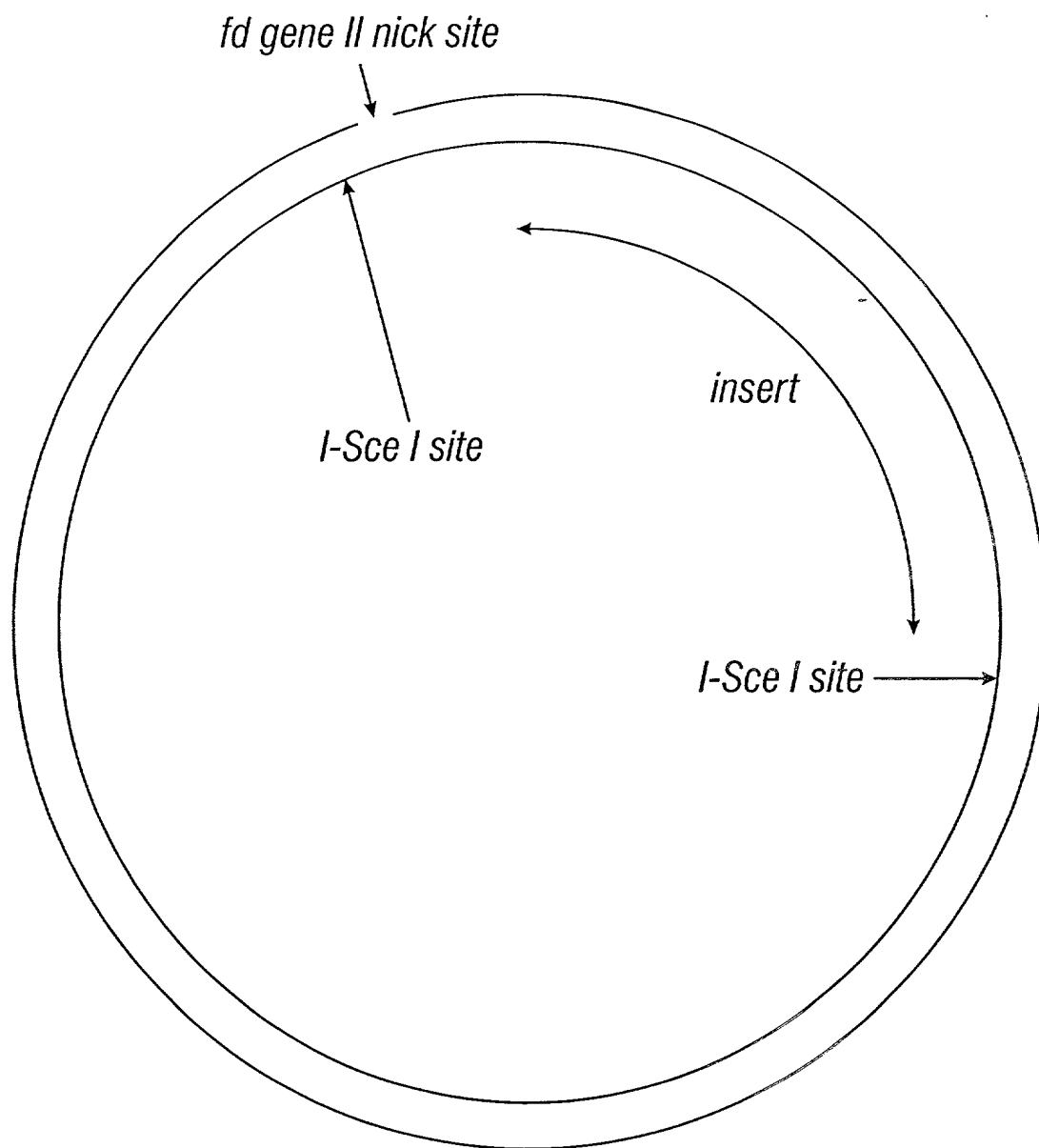


FIG. 1

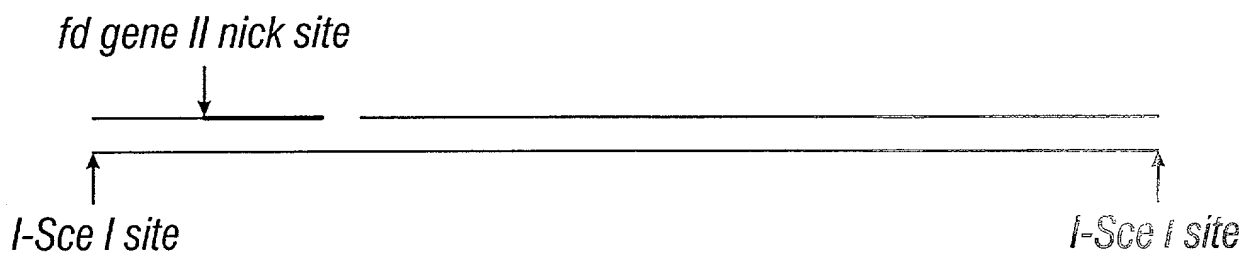


FIG. 2

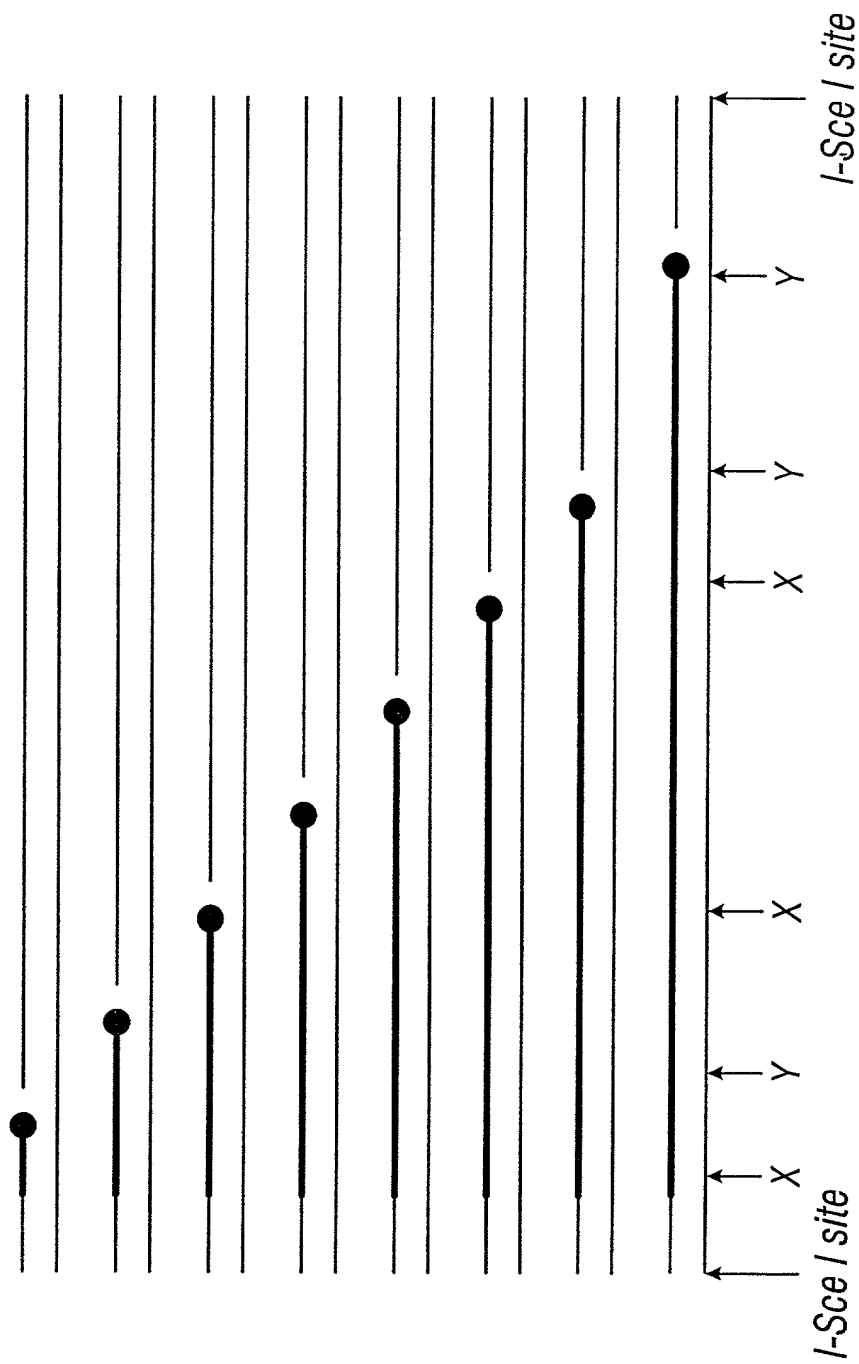


FIG. 3

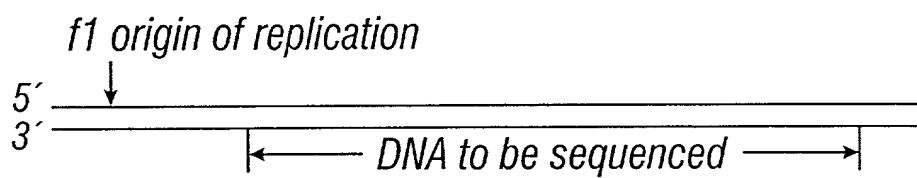


FIG. 4A



FIG. 4B



FIG. 4C



FIG. 4D



FIG. 4E

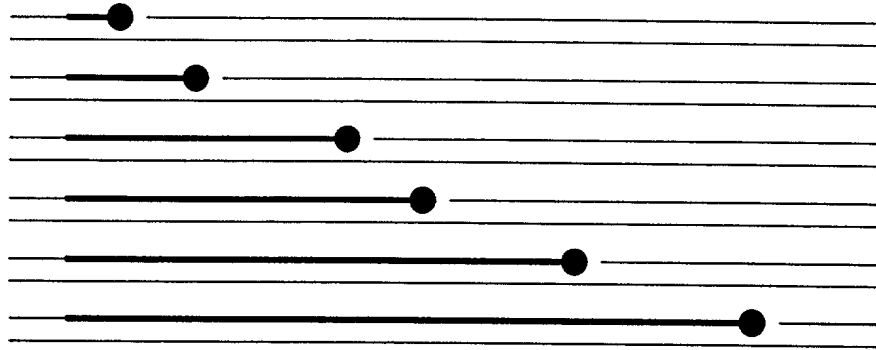


FIG. 4F

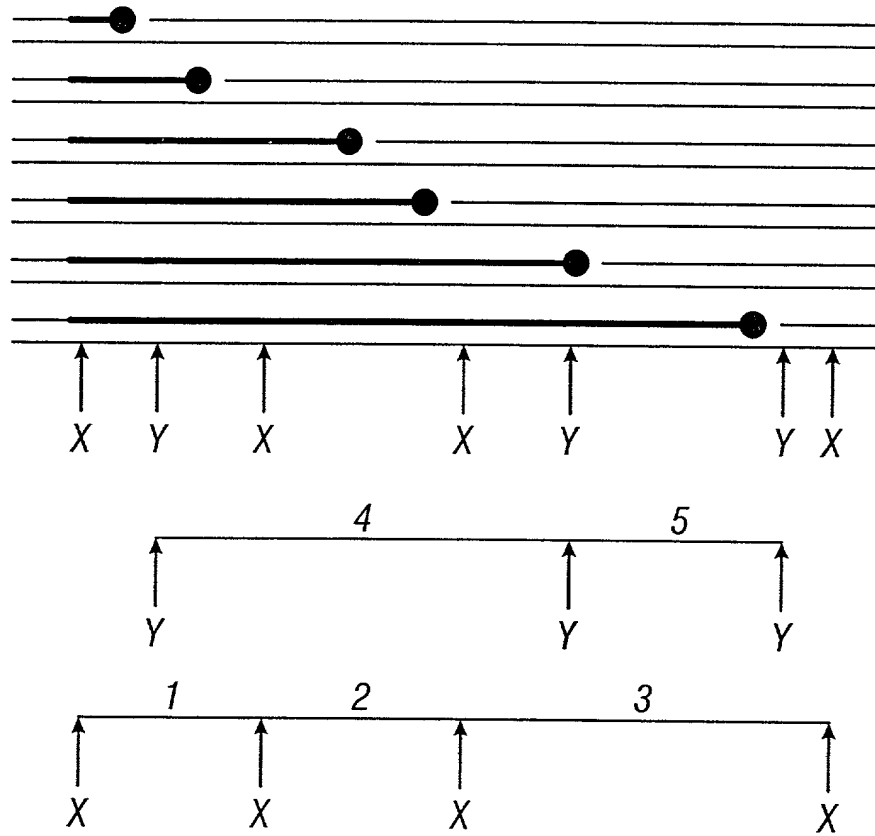


FIG. 4G

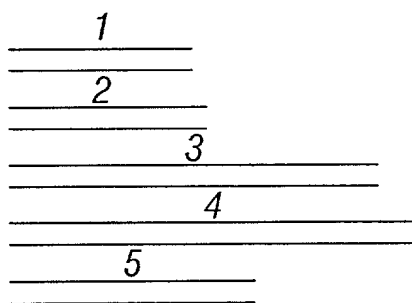


FIG. 4H

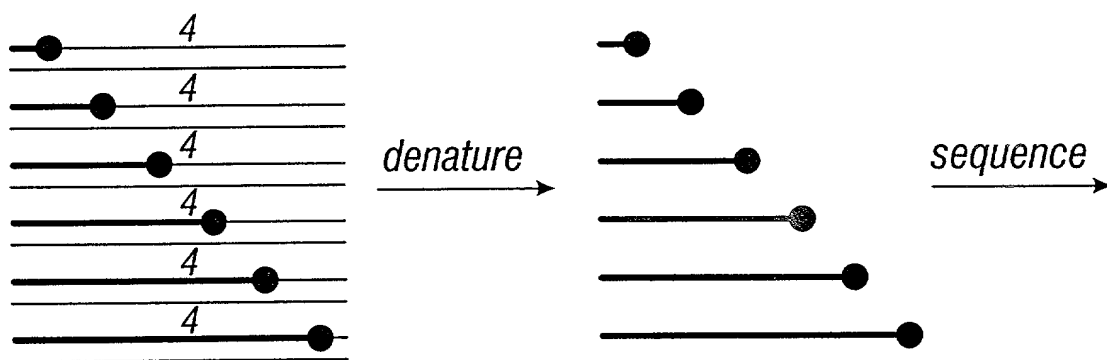


FIG. 4I

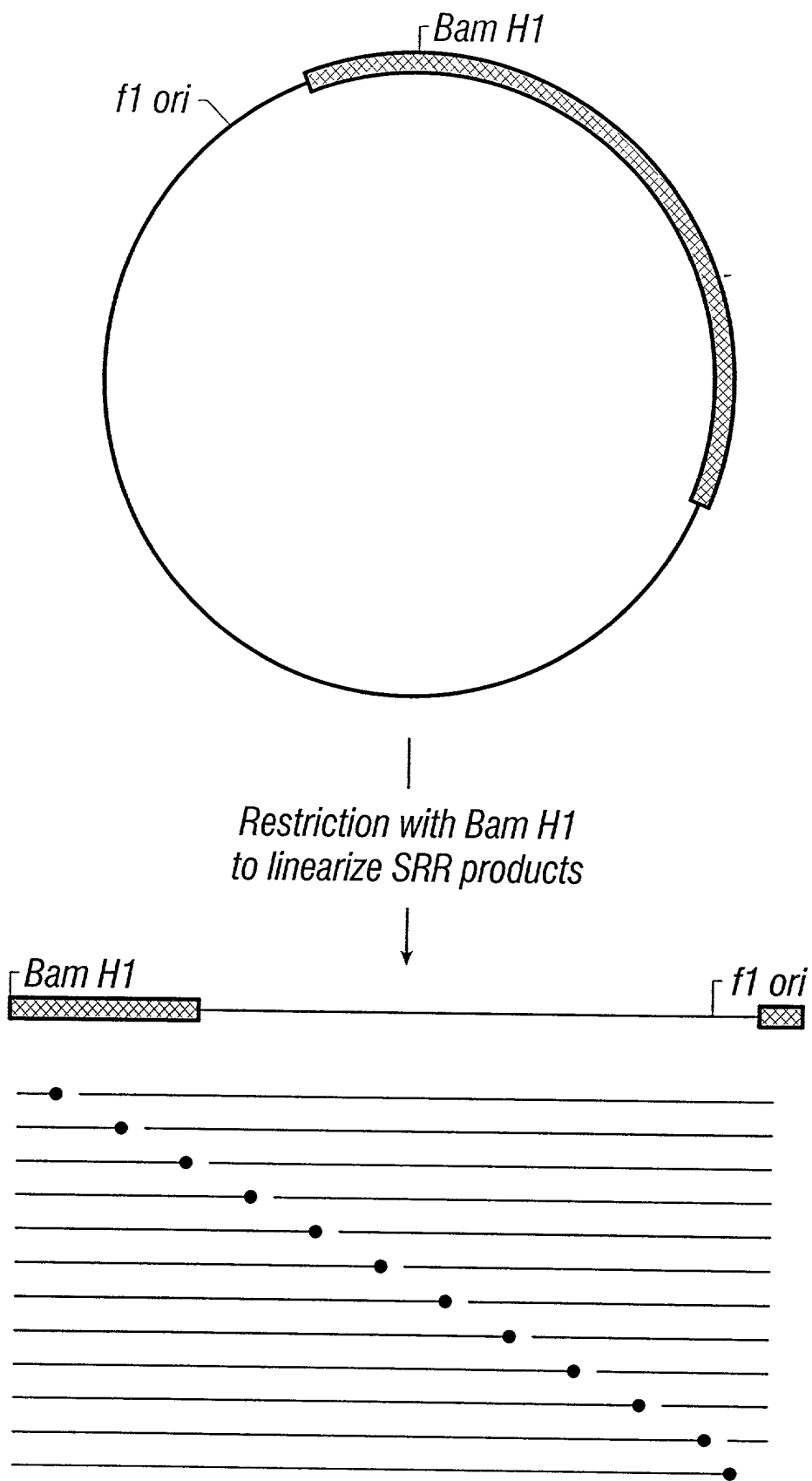


FIG. 5

5' ——— pGpGpApTpCpC
3' ——— CpCpTpApGpGp

(1)

5' ——— pG
3' ——— CpCpTpApGpGp

(2)

5' ——— pG
3' ——— CpCpTpApGpG

(3)

pGpApTpCpCpX¹pX²pX³pX⁴... 3'
G Y¹pY²pY³pY⁴p... 5'

5' ——— pGpGpApTpCpCpX¹pX²pX³pX⁴...
3' ——— CpCpTpApG GpY¹pY²pY³pY⁴p...

(4)

pGpApTpCpCpX¹pX²pX³pX⁴... 3'
Y¹pY²pY³pY⁴p... 5'

5' ——— pGpGpApTpCpCpX¹pX²pX³pX⁴...
3' ——— CpCpTpApGp Y¹pY²pY³pY⁴...

STRAND REPLACEMENT REACTION

FIG. 6

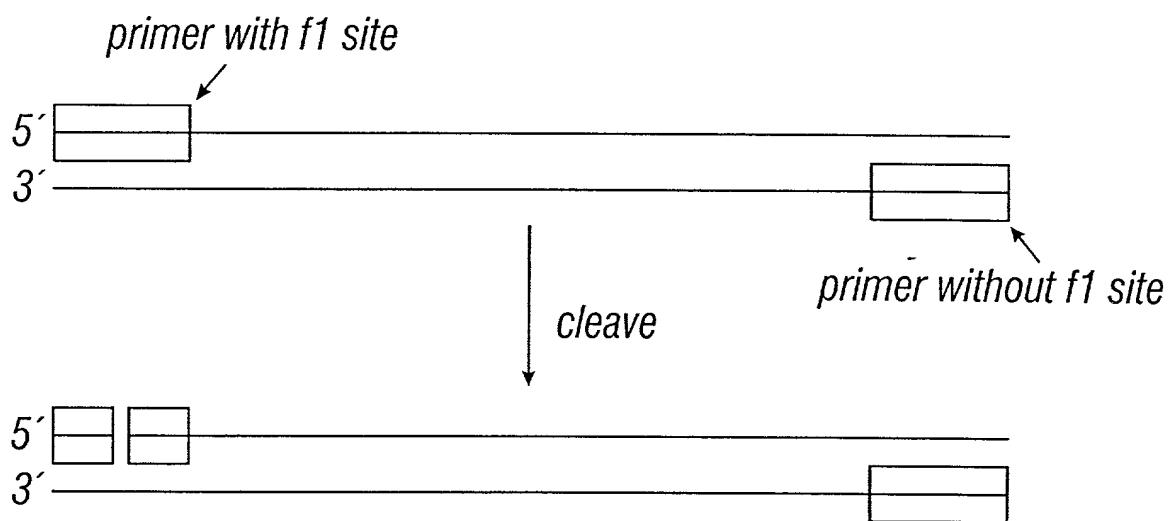


FIG. 7A

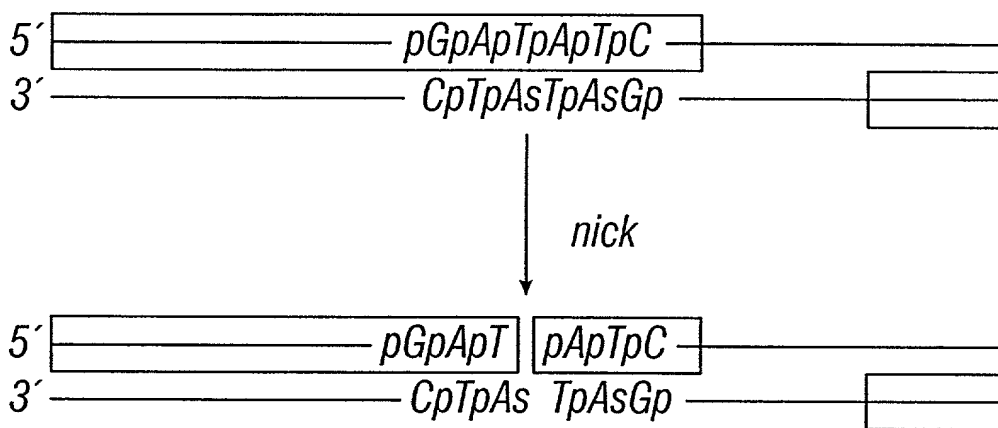


FIG. 7B

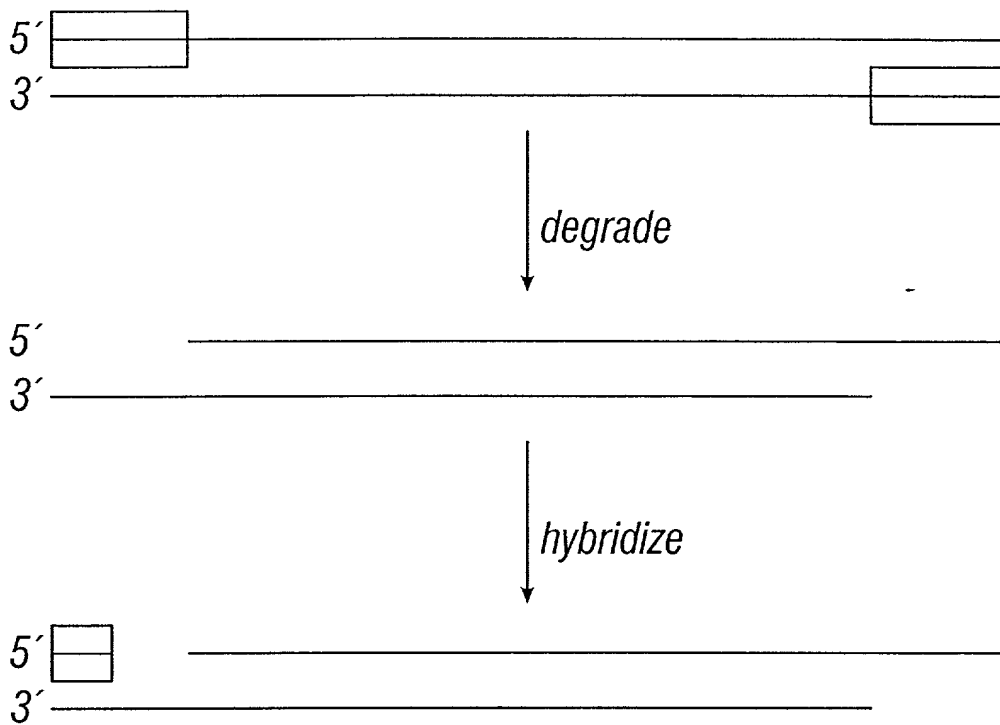


FIG. 7C

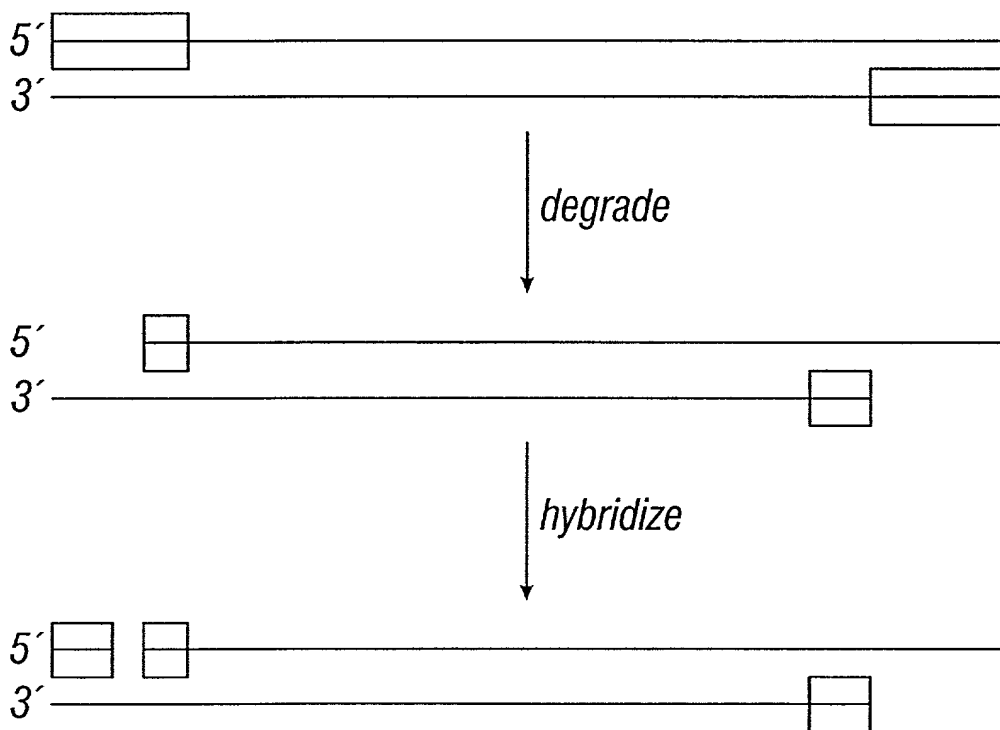


FIG. 7D

PRIMER WITH URACIL

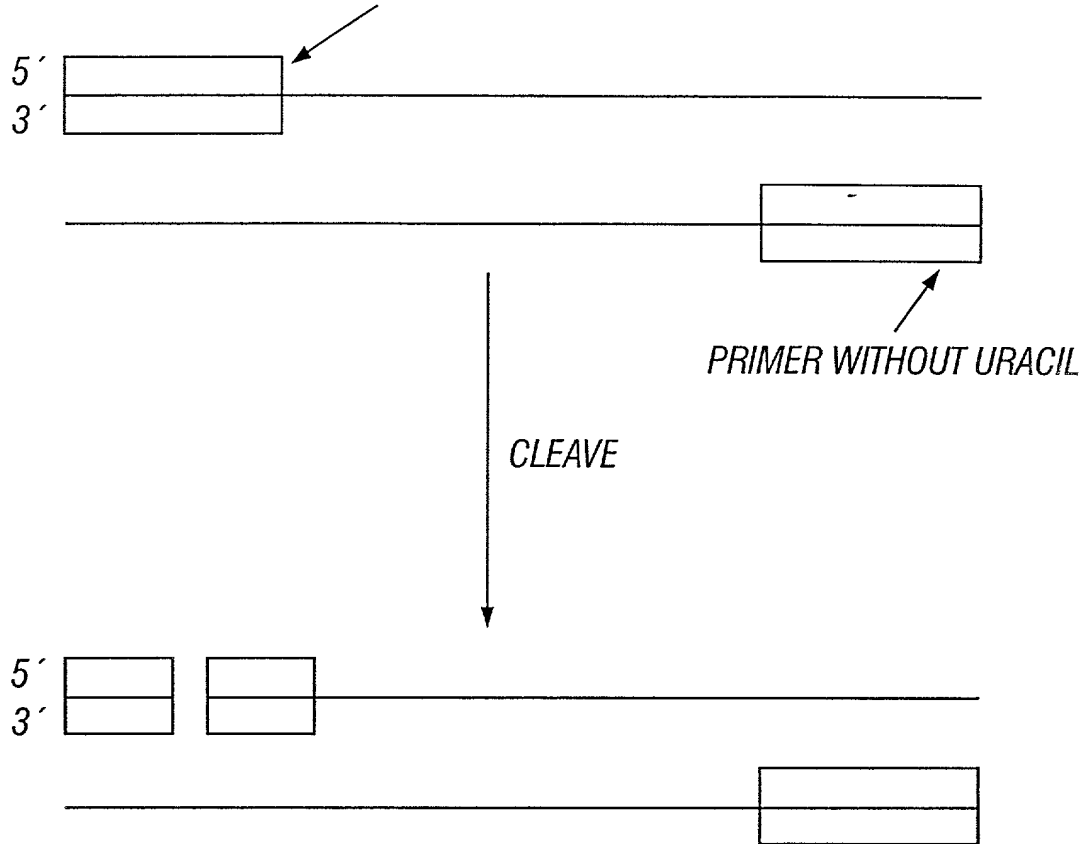


FIG. 7E

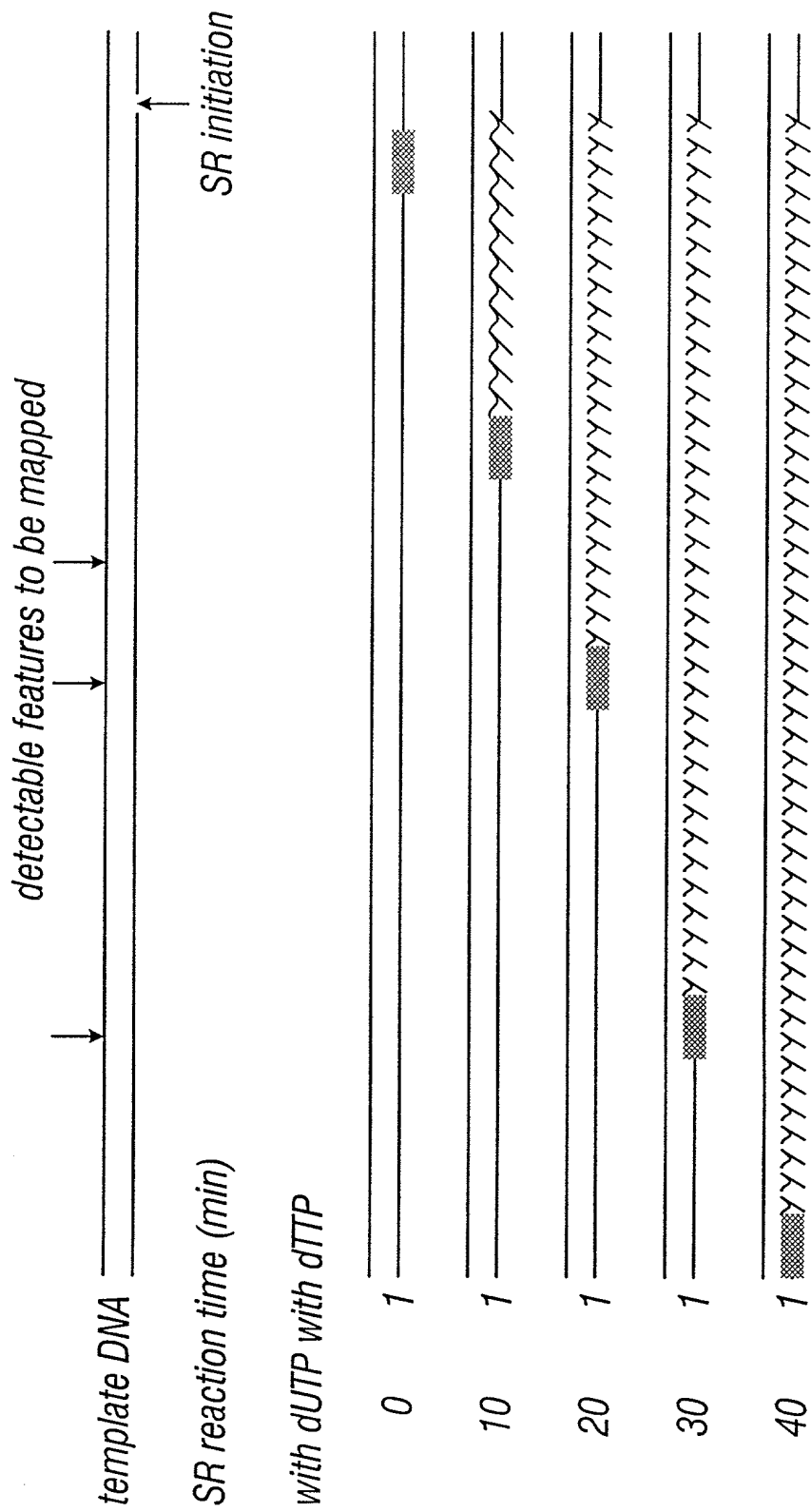


FIG. 8

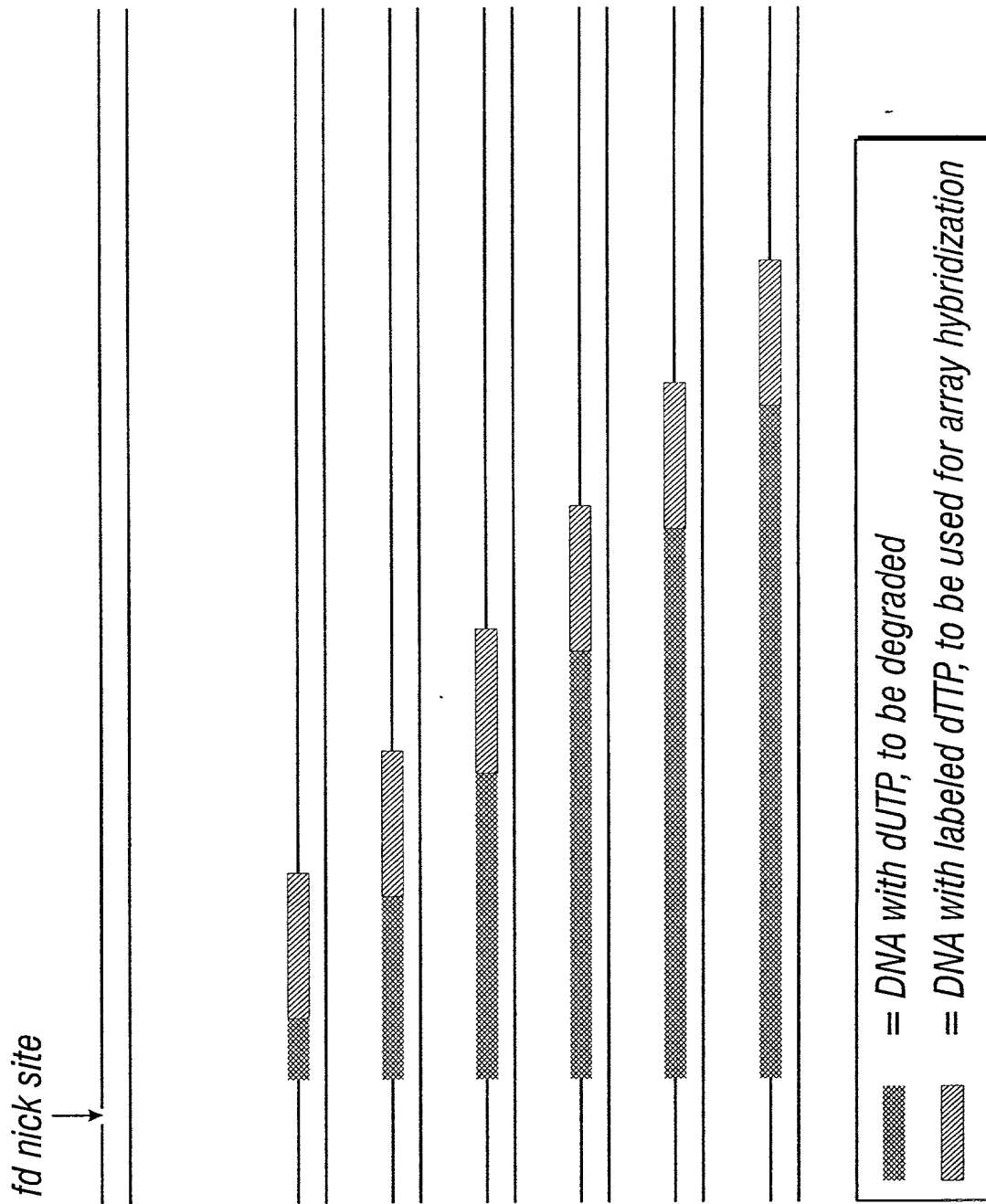


FIG. 9

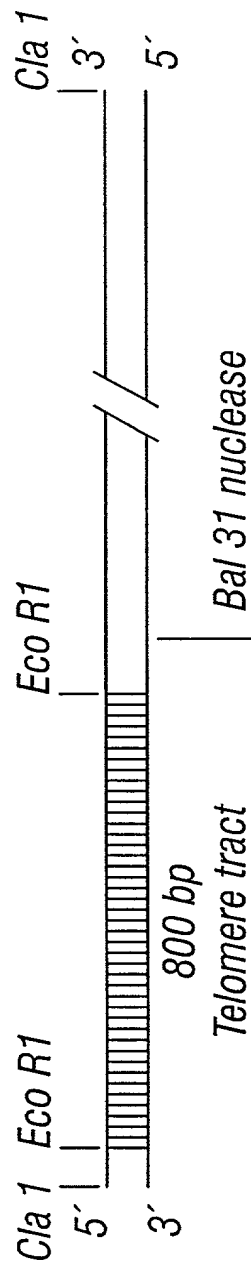


FIG. 10A

Bal 31 nuclease
T7 gene 6 exonuclease

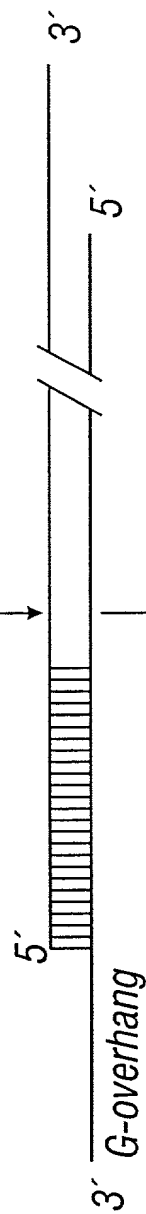


FIG. 10B

Primer
Hybridization

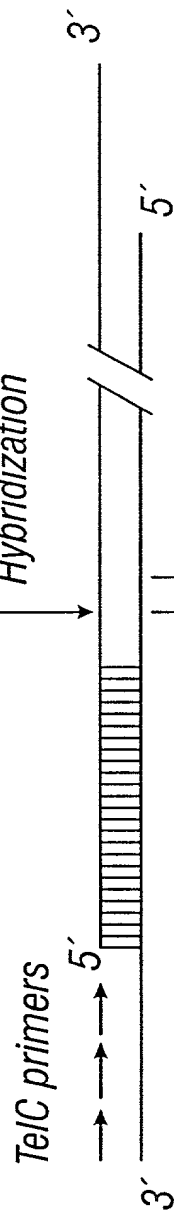


FIG. 10C

dATP, dCTP, dTTP

Tag DNA Polymerase
Extension

Nick

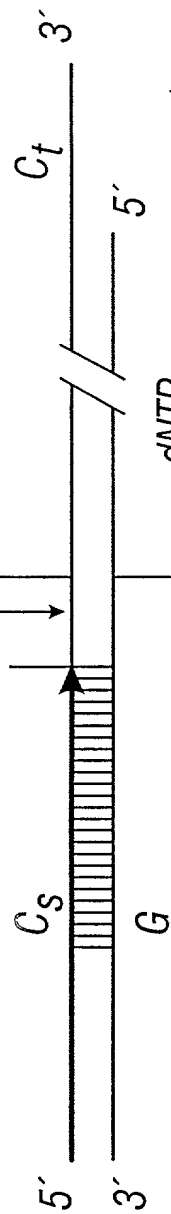


FIG. 10D

Nick

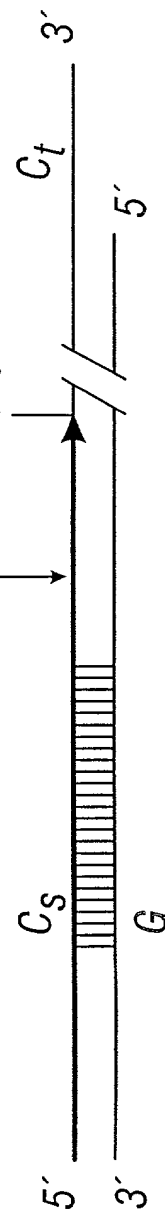


FIG. 10E

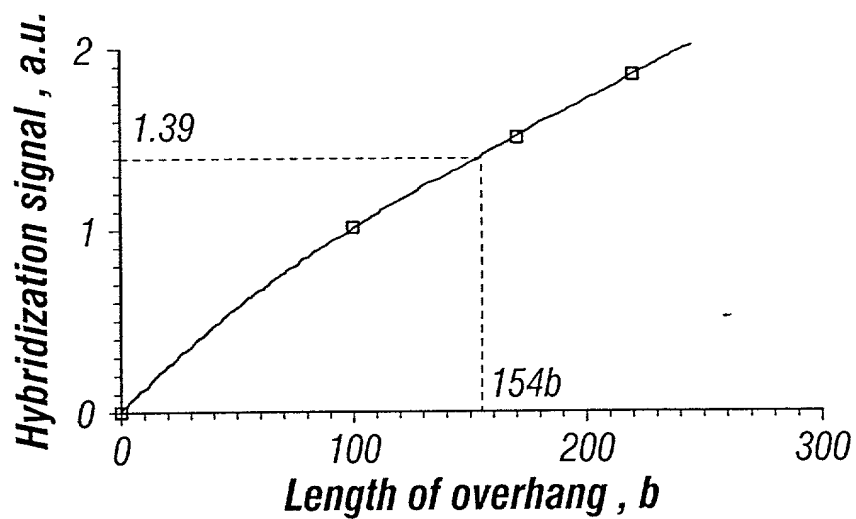
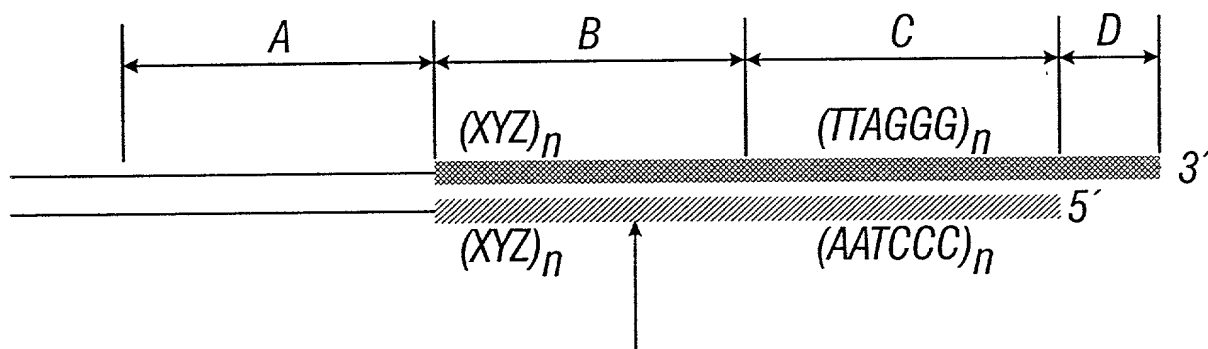


FIG. 11



site of first guanine in the C-rich strand

reaction time (min)

10



20



30

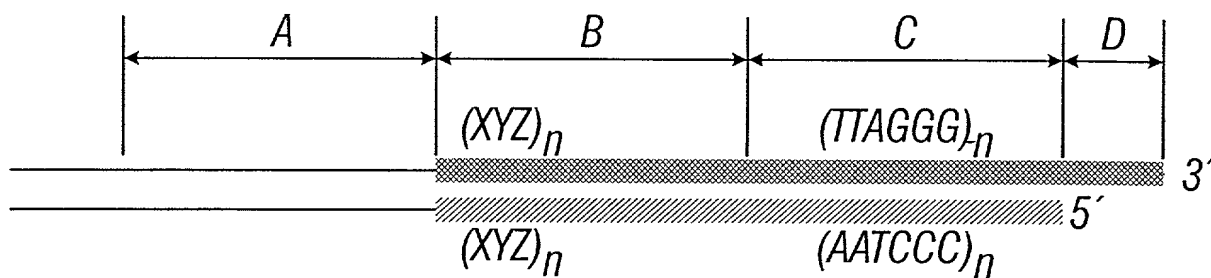


40



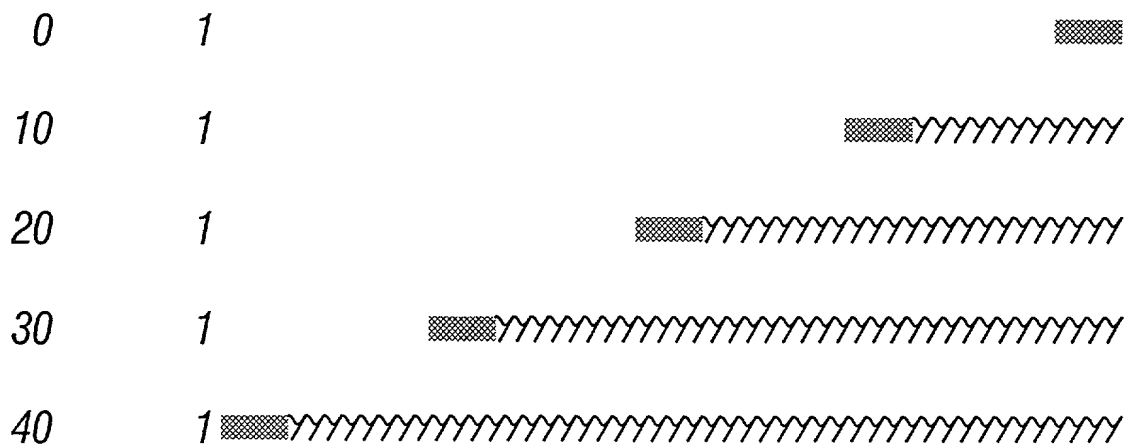
/// = DNA synthesized by SR using only dATP, dTTP, and dCTP

FIG. 12



reaction time (min)

with dUTP with dTTP



= thymidine-containing DNA synthesized by PENT
 = uridine-containing DNA synthesized by PENT

FIG. 13

FO9080" SHEF0850

^B
1 2 3 4 5 6 7 8 9 ^A
1 2 3 4 5 6 7 8 9

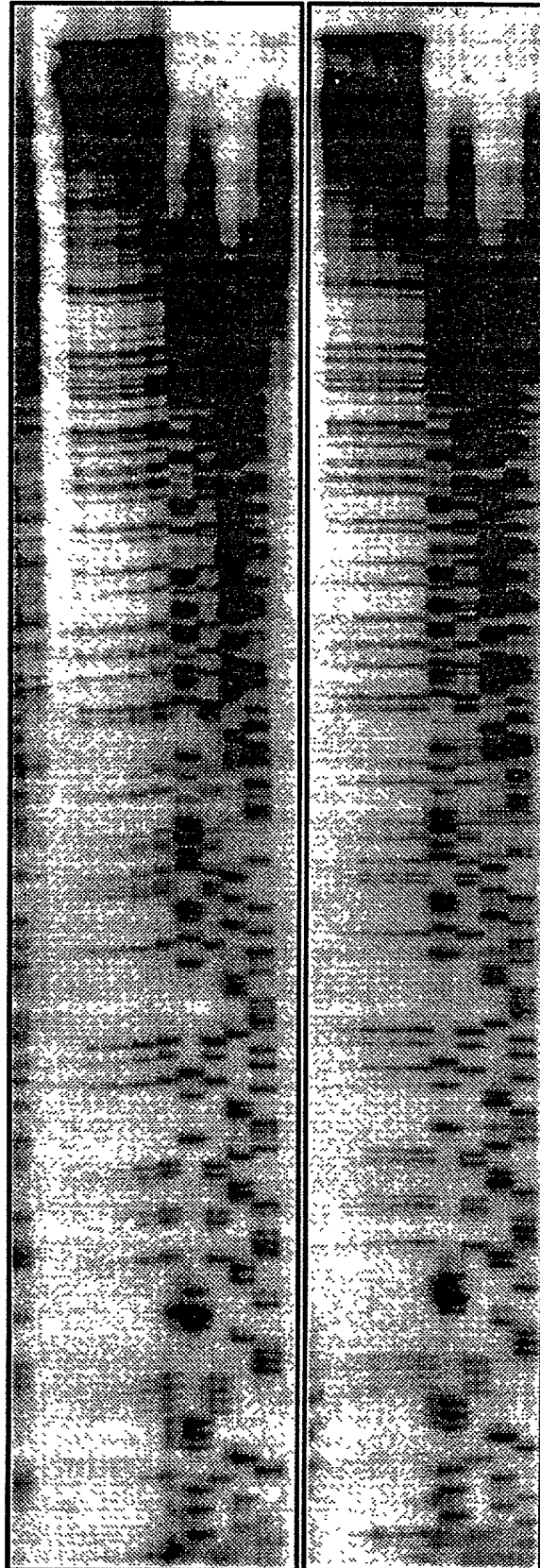


FIG. 14B

FIG. 14A

5' O-----T-A-C-T-A-T-G-G-T-T-T-A-----3' PCR-AMPLIFIED WITH DETECTION TAG AT 5' END OF
 3' -----A-T-G-A-T-A-C-C-A-A-A-T-----5' PRIMER X. NUMBERS LABEL THE 12 UNKNOWN BASES.

1 2 3 4 5 6 7 8 9 10 11 12

PRIMER X UNKNOWN DNA SEQUENCE PRIMER Y

RANDOM DEGRADATION (ONLY DAMAGED UPPER STRAND SHOWN)

| | | | |
|--------|------------------------------|--------|------------------------------|
| O----- | A-C-T-A-T-G-G-T-T-T-A----- | O----- | T-A-C-T-A-T- G-T-T-T-A----- |
| ----- | A-T-G-A-T-A-C-C-A-A-A-T----- | ----- | A-T-G-A-T-A-C-C-A-A-A-T----- |
| O----- | T- C-T-A-T-G-G-T-T-T-A----- | O----- | T-A-C-T-A-T-G- T-T-T-A----- |
| ----- | A-T-G-A-T-A-C-C-A-A-A-T----- | ----- | A-T-G-A-T-A-C-C-A-A-A-T----- |
| O----- | T-A- T-A-T-G-G-T-T-T-A----- | O----- | T-A-C-T-A-T-G-G- T-T-A----- |
| ----- | A-T-G-A-T-A-C-C-A-A-A-T----- | ----- | A-T-G-A-T-A-C-C-A-A-A-T----- |
| O----- | T-A-C- A-T-G-G-T-T-T-A----- | O----- | T-A-C-T-A-T-G-G-T- T-A----- |
| ----- | A-T-G-A-T-A-C-C-A-A-A-T----- | ----- | A-T-G-A-T-A-C-C-A-A-A-T----- |
| O----- | T-A-C-T- T-G-G-T-T-T-A----- | O----- | T-A-C-T-A-T-G-G-T-T- A----- |
| ----- | A-T-G-A-T-A-C-C-A-A-A-T----- | ----- | A-T-G-A-T-A-C-C-A-A-A-T----- |
| O----- | T-A-C-T-A- G-G-T-T-T-A----- | O----- | T-A-C-T-A-T-G-G-T-T-T- ----- |
| ----- | A-T-G-A-T-A-C-C-A-A-A-T----- | ----- | A-T-G-A-T-A-C-C-A-A-A-T----- |

| |
|-----|
| 15A |
| 15B |

FIG. 15

EXPOSE 3'OH AT DAMAGE SITES

| | | | |
|--------|------------------------------|--------|------------------------------|
| O----- | A-C-T-A-T-G-G-T-T-T-A----- | O----- | T-A-C-T-A-T- G-T-T-T-A----- |
| ----- | A-T-G-A-T-A-C-C-A-A-A-T----- | ----- | A-T-G-A-T-A-C-C-A-A-A-T----- |
| O----- | T C-T-A-T-G-G-T-T-T-A----- | O----- | T-A-C-T-A-T-G T-T-T-A----- |
| ----- | A-T-G-A-T-A-C-C-A-A-A-T----- | ----- | A-T-G-A-T-A-C-C-A-A-A-T----- |
| O----- | T-A T-A-T-G-G-T-T-T-A----- | O----- | T-A-C-T-A-T-G-G T-T-A----- |
| ----- | A-T-G-A-T-A-C-C-A-A-A-T----- | ----- | A-T-G-A-T-A-C-C-A-A-A-T----- |
| O----- | T-A-C A-T-G-G-T-T-T-A----- | O----- | T-A-C-T-A-T-G-G-T T-A----- |
| ----- | A-T-G-A-T-A-C-C-A-A-A-T----- | ----- | A-T-G-A-T-A-C-C-A-A-A-T----- |
| O----- | T-A-C-T T-G-G-T-T-T-A----- | O----- | T-A-C-T-A-T-G-G-T-T A----- |
| ----- | A-T-G-A-T-A-C-C-A-A-A-T----- | ----- | A-T-G-A-T-A-C-C-A-A-A-T----- |
| O----- | T-A-C-T-A G-G-T-T-T-A----- | O----- | T-A-C-T-A-T-G-G-T-T-T ----- |
| ----- | A-T-G-A-T-A-C-C-A-A-A-T----- | ----- | A-T-G-A-T-A-C-C-A-A-A-T----- |

FIG. 15A

INCORPORATE BIOTINYLATED DDTP AT POSITIONS
OPPOSITE ADENINE IN TEMPLATE STRAND

| | |
|------------------------------------|------------------------------------|
| O-----T-A-C-T-A-T-G-G-T-T-T-A----- | O-----T-A-C-T-A-T G-T-T-T-A----- |
| -----A-T-G-A-T-A-C-C-A-A-A-T----- | -----A-T-G-A-T-A-C-C-A-A-A-T----- |
| O-----T C-T-A-T-G-G-T-T-T-A----- | O-----T-A-C-T-A-T-G T-T-T-A----- |
| -----A-T-G-A-T-A-C-C-A-A-A-T----- | -----A-T-G-A-T-A-C-C-A-A-A-T----- |
| O-----T-A T-A-T-G-G-T-T-T-A----- | O-----T-A-C-T-A-T-G-G-T-T-T-A----- |
| -----A-T-G-A-T-A-C-C-A-A-A-T----- | -----A-T-G-A-T-A-C-C-A-A-A-T----- |
| O-----T-A-C-T.A-T-G-G-T-T-T-A----- | O-----T-A-C-T-A-T-G-G-T-T.T-A----- |
| -----A-T-G-A-T-A-C-C-A-A-A-T----- | -----A-T-G-A-T-A-C-C-A-A-A-T----- |
| O-----T-A-C-T T-G-G-T-T-T-A----- | O-----T-A-C-T-A-T-G-G-T-T-T.A----- |
| -----A-T-G-A-T-A-C-C-A-A-A-T----- | -----A-T-G-A-T-A-C-C-A-A-A-T----- |
| O-----T-A-C-T-A.T.G-G-T-T-T-A----- | O-----T-A-C-T-A-T-G-G.T-T-T----- |
| -----A-T-G-A-T-A-C-C-A-A-A-T----- | -----A-T-G-A-T-A-C-C-A-A-A-T----- |

IMMOBILIZE BIOTINYLATED STRANDS AND REMOVE
NON-BIOTINYLATED STRANDS

| | |
|--------------------|------------------------------|
| O-----T. | O-----T-A-C-T-A-T-G-G-T. |
| O-----T-A-C-T. | O-----T-A-C-T-A-T-G-G-T-T. |
| O-----T-A-C-T-A-T. | O-----T-A-C-T-A-T-G-G-T-T-T. |

RELEASE BIOTINYLATED STRANDS, SEPARATE BY
ELECTROPHORESIS, AND DETECT TAGGED PRIMERS
(DARK BARS REPRESENT POSITIONS OF THYMINE)

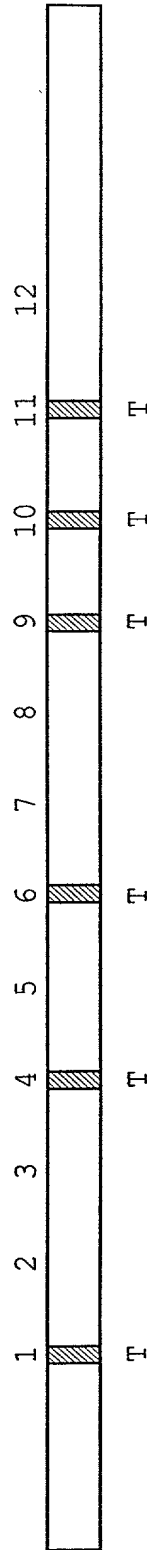


FIG. 15B

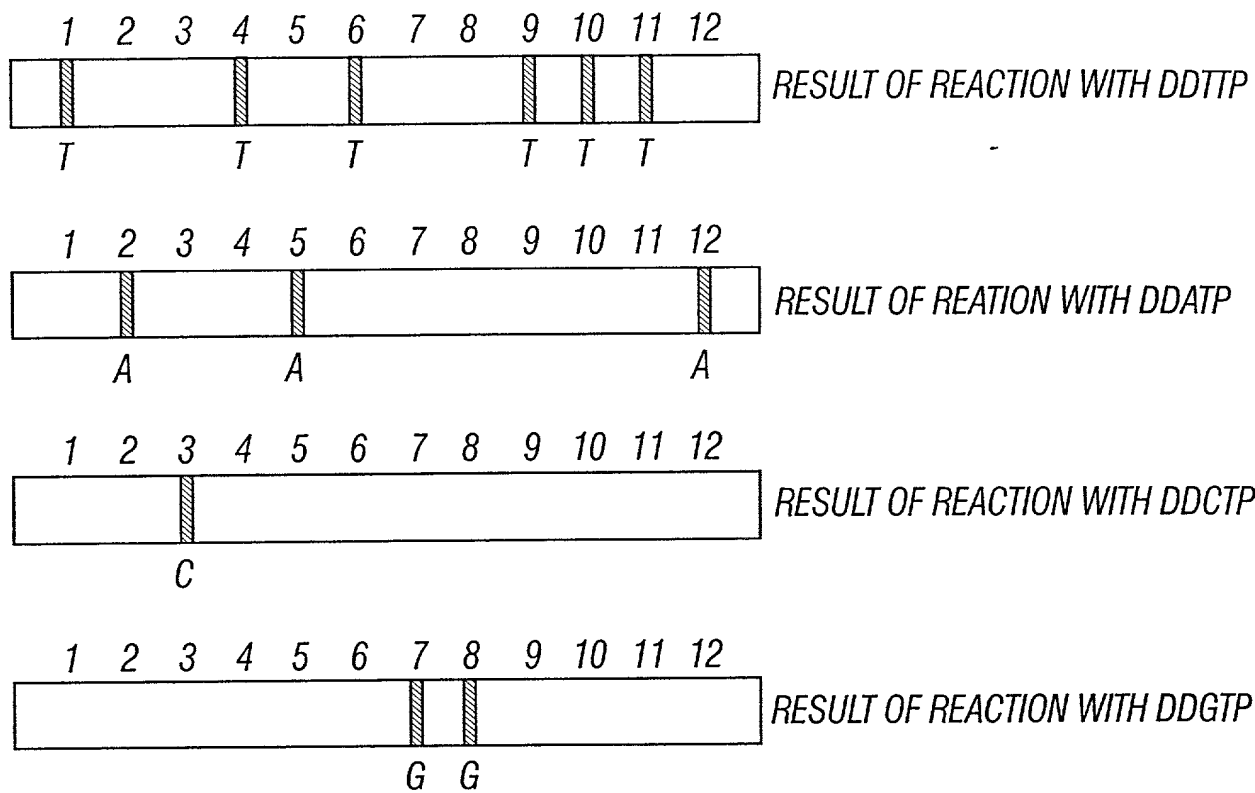


FIG. 16A

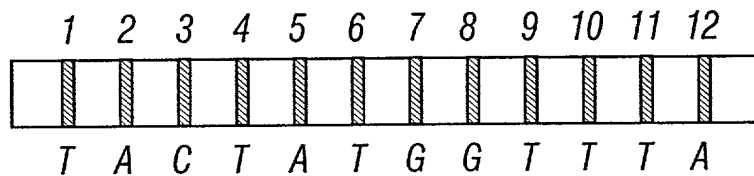
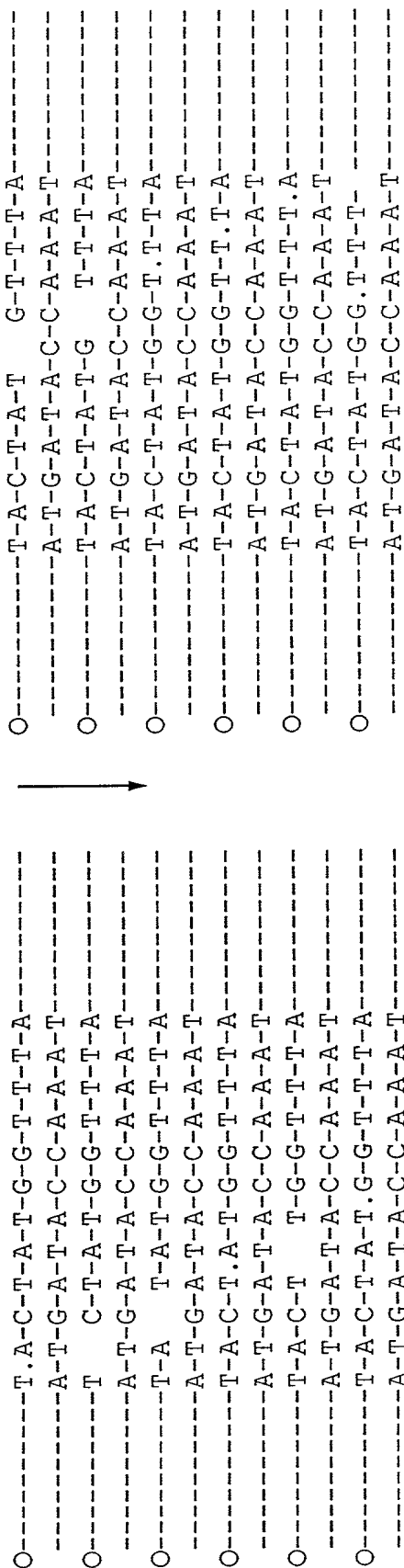
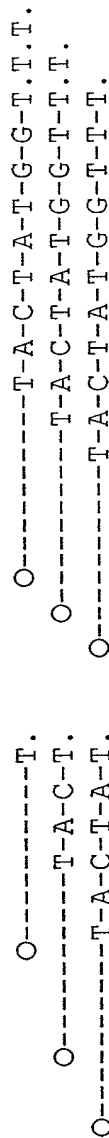


FIG. 16B

incorporate biotinylated ddTTP at positions
opposite adenine in template strand



immobilize biotinylated strands and remove
non-biotinylated strands



release biotinylated strands, separate by
electrophoresis, and detect tagged primers
(dark bars represent positions of terminal thymine)

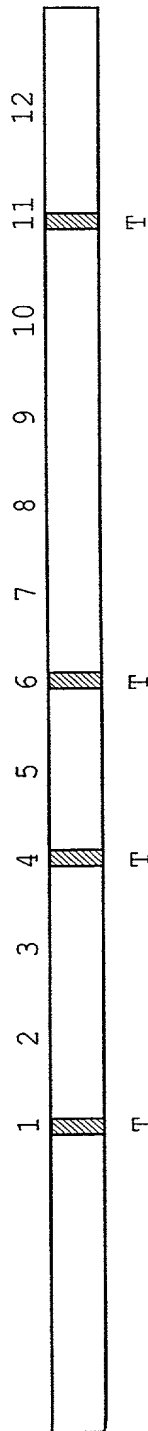


FIG. 17B

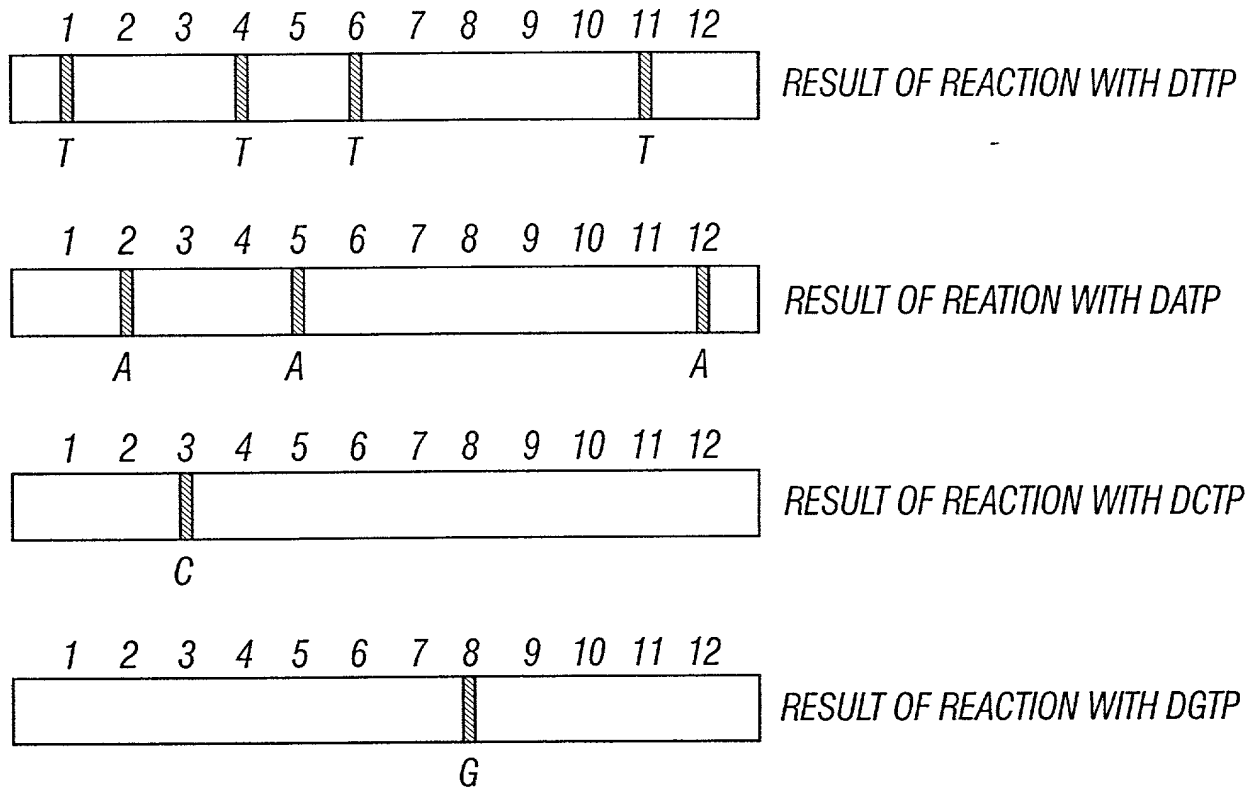


FIG. 18A

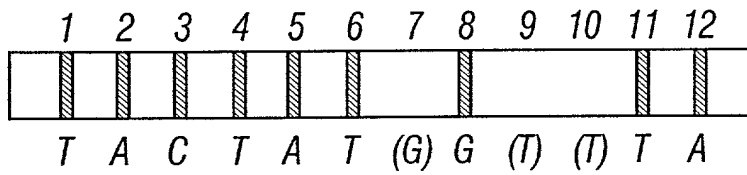


FIG. 18B

5' O-----T-A-C-T-A-T-G-G-T-T-T-A-----3' PCR-AMPLIFIED IMMOBILIZED AT 5' END OF PRIMER
 3' -----A-T-G-A-T-A-C-C-A-A-A-T-----5' X. NUMBERS LABEL THE 12 UNKNOWN BASES.
 1 2 3 4 5 6 7 8 9 10 11 12
 PRIMER X UNKNOWN DNA SEQUENCE PRIMER Y

RANDOM DEGRADATION (ONLY DAMAGED UPPER STRAND SHOWN)

O-----T-A-C-T-A-T- G-T-T-T-A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T-A-C-T-A-T-G- T-T-T-A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T-A-C-T-A-T-G-G- T-T-A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T-A-C-T-A-T-G-G-T- T-A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T-A-C-T-A-T-G-G-T-T- A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T-A-C-T-A-T-G-G-T-T-T-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----

EXPOSE 3'OH AT DAMAGE SITES

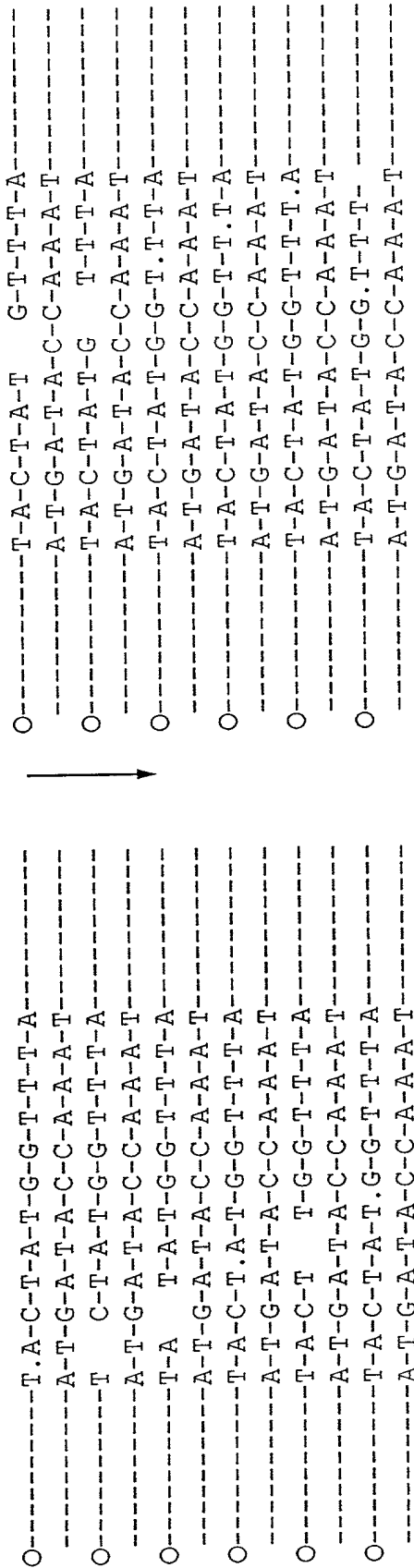
O-----T-A-C-T-A-T G-T-T-T-A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T-A-C-T-A-T-G T-T-T-A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T-A-C-T-A-T-G-G T-T-A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T-A-C-T-A-T-G-G-T T-A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T-A-C-T-A-T-G-G-T-T A-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----
 O-----T-A-C-T-A-T-G-G-T-T-T-----
 -----A-T-G-A-T-A-C-C-A-A-A-T-----

19A
19B

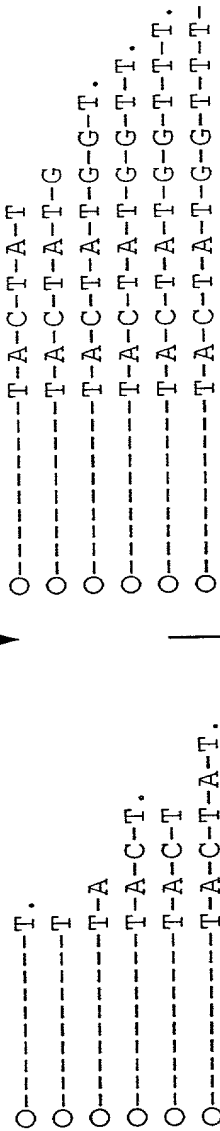
FIG. 19A

FIG. 19

INCORPORATE TAGGED DDTTP AT POSITIONS
OPPOSITE ADENINE IN TEMPLATE STRAND



DENATURE AND WASH TO REMOVE ALL STRANDS THAT ARE
NOT TAGGED AT 5' END



MOBILIZE STRANDS, SEPARATE BY
ELECTROPHORESIS, AND DETECT TAGGED BASES
(DARK BARS REPRESENT POSITIONS OF THYMINE)

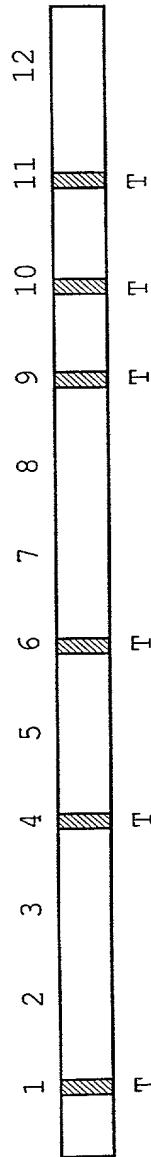


FIG. 19B

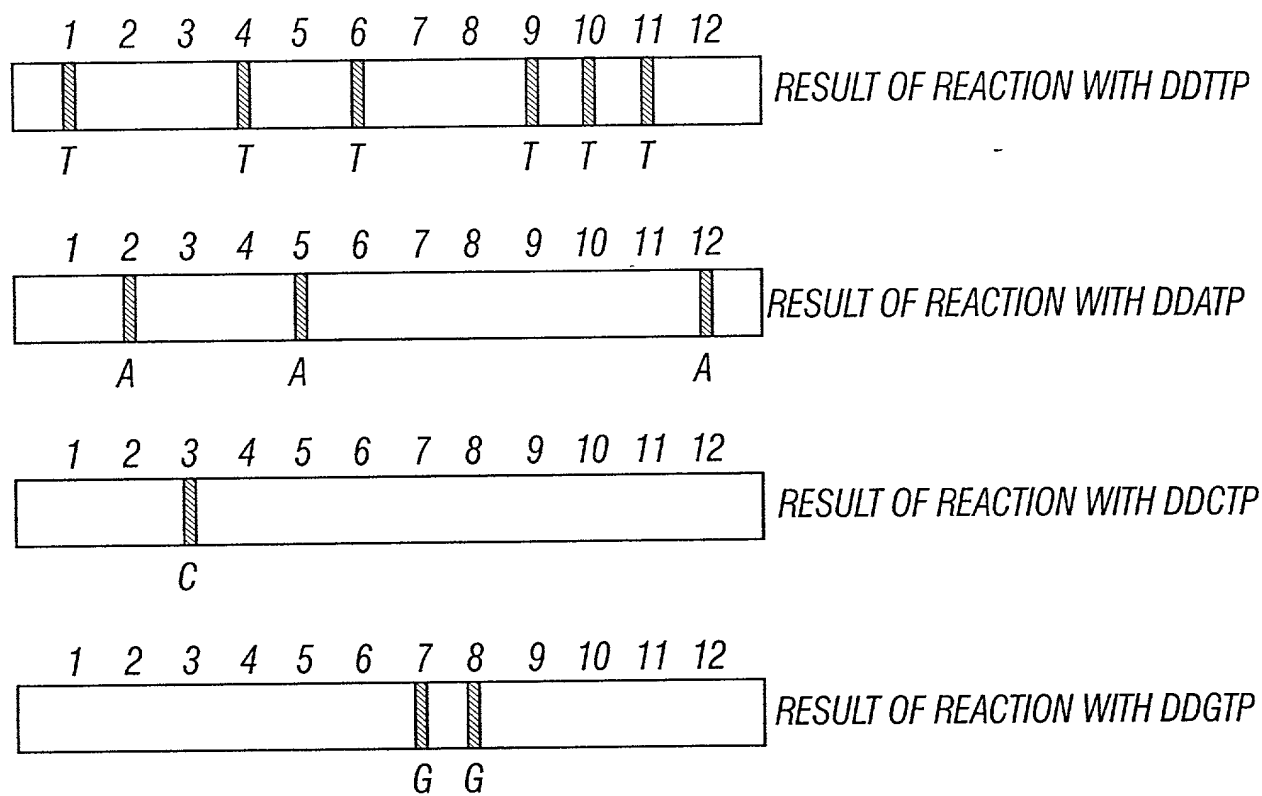


FIG. 20A

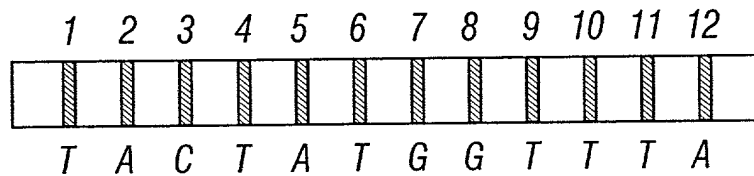


FIG. 20B

PCR amplify, immobilize, and expose OH
at random sites as in Fig. 5.

| | |
|-----------------------------------|------------------------------------|
| O-----A-C-T-A-T-G-G-T-T-T-A----- | O-----T-A-C-T-A-T G-T-T-T-A----- |
| -----A-T-G-A-T-A-C-C-A-A-A-T----- | -----A-T-G-A-T-A-C-C-A-A-A-T----- |
| O-----C-T-A-T-G-G-T-T-T-A----- | O-----T-A-C-T-A-T-G T-T-T-A----- |
| -----A-T-G-A-T-A-C-C-A-A-A-T----- | -----A-T-G-A-T-A-C-C-A-A-A-T----- |
| O-----T-A T-A-T-G-G-T-T-T-A----- | O-----T-A-C-T-A-T-G-G T-T-A----- |
| -----A-T-G-A-T-A-C-C-A-A-A-T----- | -----A-T-G-A-T-A-C-C-A-A-A-T----- |
| O-----T-A-C A-T-G-G-T-T-T-A----- | O-----T-A-C-T-A-T-G-G-T T-A----- |
| -----A-T-G-A-T-A-C-C-A-A-A-T----- | -----A-T-G-A-T-A-C-C-A-A-A-T----- |
| O-----T-A-C-T T-G-G-T-T-T-A----- | O-----T-A-C-T-A-T-G-G-T-T A----- |
| -----A-T-G-A-T-A-C-C-A-A-A-T----- | -----A-T-G-A-T-A-C-C-A-A-A-T----- |
| O-----T-A-C-T-A G-G-T-T-T-A----- | O-----T-A-C-T-A-T-G-G-T-T-T ----- |
| -----A-T-G-A-T-A-C-C-A-A-A-T----- | -----A-T-G-A-T-A-C-C-A-A-A-T----- |

Block ends opposite T, G' & C with ddATP, ddGTP, ddCTP
(shown in bold letters), remove ddNTPs, then add dTTP.

| | |
|------------------------------------|------------------------------------|
| O-----T-A-C-T-A-T-G-G-T-T-T-A----- | O-----T-A-C-T-A-T-G-G-T-T-T-A----- |
| -----A-T-G-A-T-A-C-C-A-A-A-T----- | -----A-T-G-A-T-A-C-C-A-A-A-T----- |
| O-----T-A-C-T-A-T-G-G-T-T-T-A----- | O-----T-A-C-T-A-T-G-G-T-T-T-A----- |
| -----A-T-G-A-T-A-C-C-A-A-A-T----- | -----A-T-G-A-T-A-C-C-A-A-A-T----- |
| O-----T-A-C-T-A-T-G-G-T-T-T-A----- | O-----T-A-C-T-A-T-G-G-T-T-T-A----- |
| -----A-T-G-A-T-A-C-C-A-A-A-T----- | -----A-T-G-A-T-A-C-C-A-A-A-T----- |
| O-----T-A-C-T-A-T-G-G-T-T-T-A----- | O-----T-A-C-T-A-T-G-G-T-T-T-A----- |
| -----A-T-G-A-T-A-C-C-A-A-A-T----- | -----A-T-G-A-T-A-C-C-A-A-A-T----- |
| O-----T-A-C-T-A-T-G-G-T-T-T-A----- | O-----T-A-C-T-A-T-G-G-T-T-T-A----- |
| -----A-T-G-A-T-A-C-C-A-A-A-T----- | -----A-T-G-A-T-A-C-C-A-A-A-T----- |

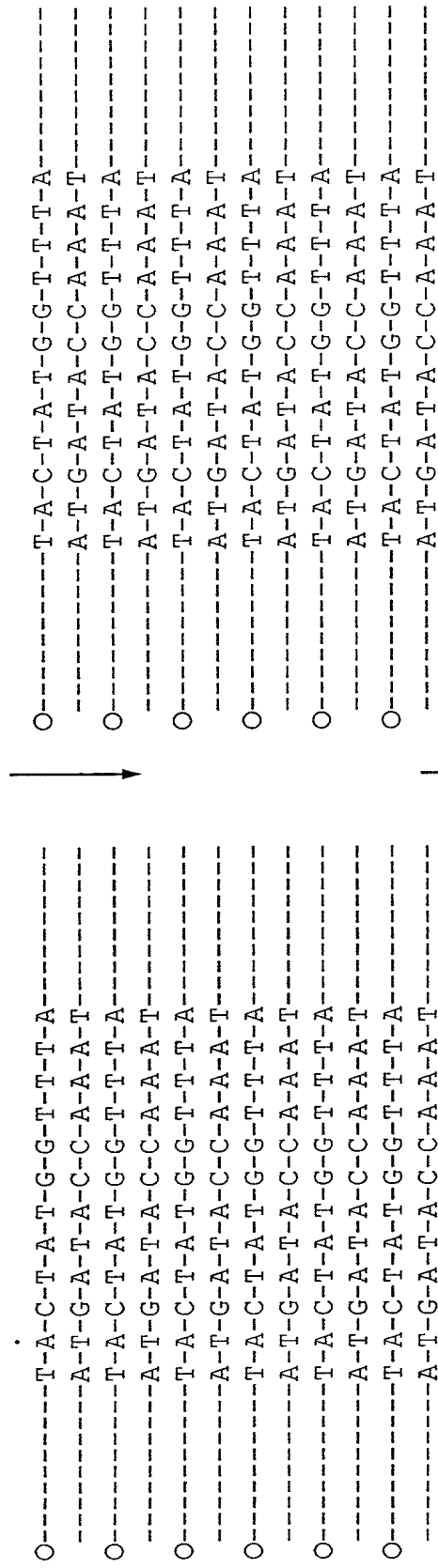
| |
|-----|
| 21A |
| 21B |

FIG. 21A

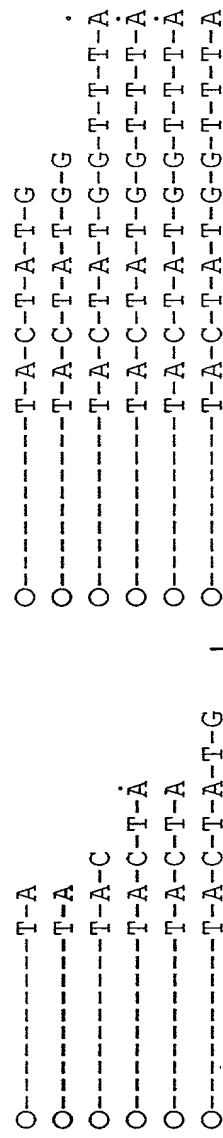
FIG. 21

[illegible]

Block ends opposite A, G & C with ddTTP, ddGTP, ddCTP (shown in bold letters), remove ddNTPs, then add tagged ddATP.



Denature and wash to remove all strands that are not tagged at 5' end.



Mobilize strands, separate by electrophoresis, and detect tagged bases (dark bars).

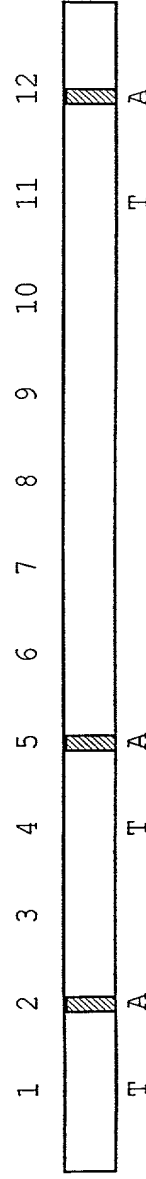


FIG. 21B

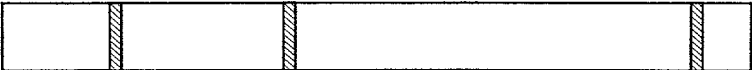




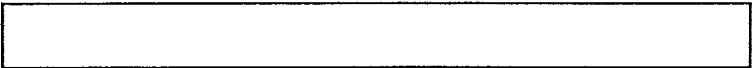

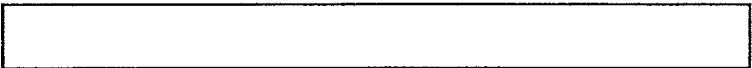
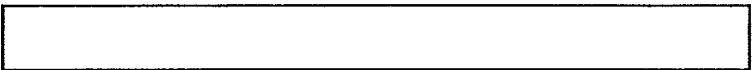
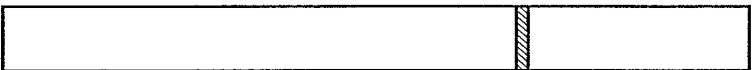


| 1 2 3 4 5 6 7 8 9 10 11 12 | | <i>BASES WALKED</i> |
|---|-----|---------------------|
|  | T/A | - |
|  | T/C | - |
|  | T/G | - |
|  | A/T | - |
|  | A/C | - |
|  | A/G | - |
|  | C/T | - |
|  | C/A | - |
|  | C/G | - |
|  | G/T | - |
|  | G/A | - |
|  | G/C | - |

FIG. 22A

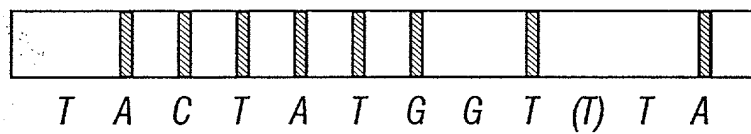


FIG. 22B

| | | | |
|--------|------------------------------|--------|------------------------------|
| O----- | A-C-T-A-T-G-G-T-T-T-A----- | O----- | T-A-C-T-A-T----- |
| ----- | A-T-G-A-T-A-C-C-A-A-A-T----- | ----- | A-T-G-A-T-A-C-C-A-A-A-T----- |
| O----- | T----- | O----- | T-A-C-T-A-T-G----- |
| ----- | A-T-G-A-T-A-C-C-A-A-A-T----- | ----- | A-T-G-A-T-A-C-C-A-A-A-T----- |
| O----- | T-A----- | O----- | T-A-C-T-A-T-G----- |
| ----- | A-T-G-A-T-A-C-C-A-A-A-T----- | ----- | A-T-G-A-T-A-C-C-A-A-A-T----- |
| O----- | T-A----- | O----- | T-A-C-T-A-T-G----- |
| ----- | A-T-G-A-T-A-C-C-A-A-A-T----- | ----- | A-T-G-A-T-A-C-C-A-A-A-T----- |
| O----- | T-A-C----- | O----- | T-A-C-T-A-T-G-G-T----- |
| ----- | A-T-G-A-T-A-C-C-A-A-A-T----- | ----- | A-T-G-A-T-A-C-C-A-A-A-T----- |
| O----- | T-A-C-T----- | O----- | T-A-C-T-A-T-G-G-T----- |
| ----- | A-T-G-A-T-A-C-C-A-A-A-T----- | ----- | A-T-G-A-T-A-C-C-A-A-A-T----- |
| O----- | T-A-C-T-A----- | O----- | T-A-C-T-A-T-G-G-T-T----- |
| ----- | A-T-G-A-T-A-C-C-A-A-A-T----- | ----- | A-T-G-A-T-A-C-C-A-A-A-T----- |

Block ends opposite T, G & C with ddATP, ddGTP, ddCTP (shown in bold letters), remove ddNTPs, then add dTTP.

[illegible]

235

FIG. 23

FIG. 23A

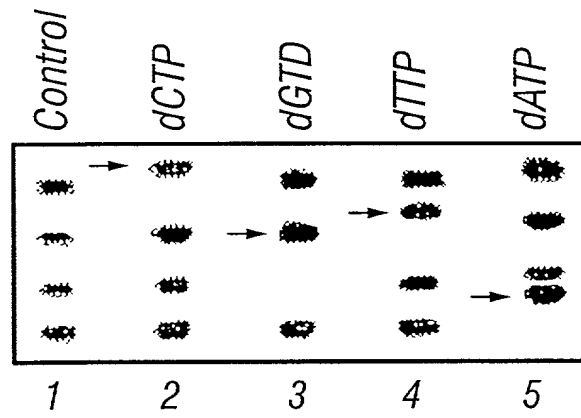


FIG. 24

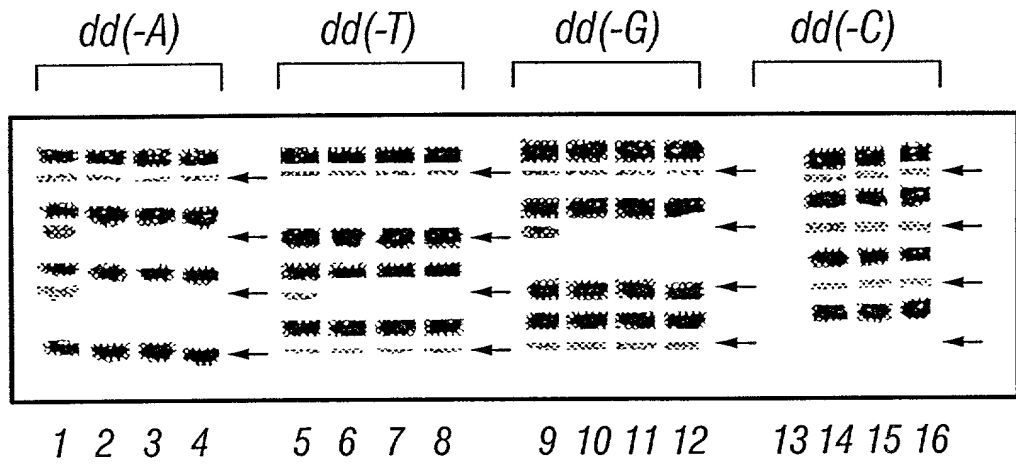


FIG. 25

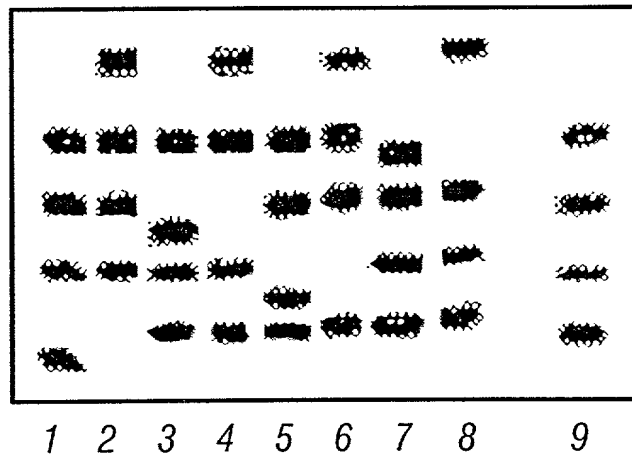


FIG. 26

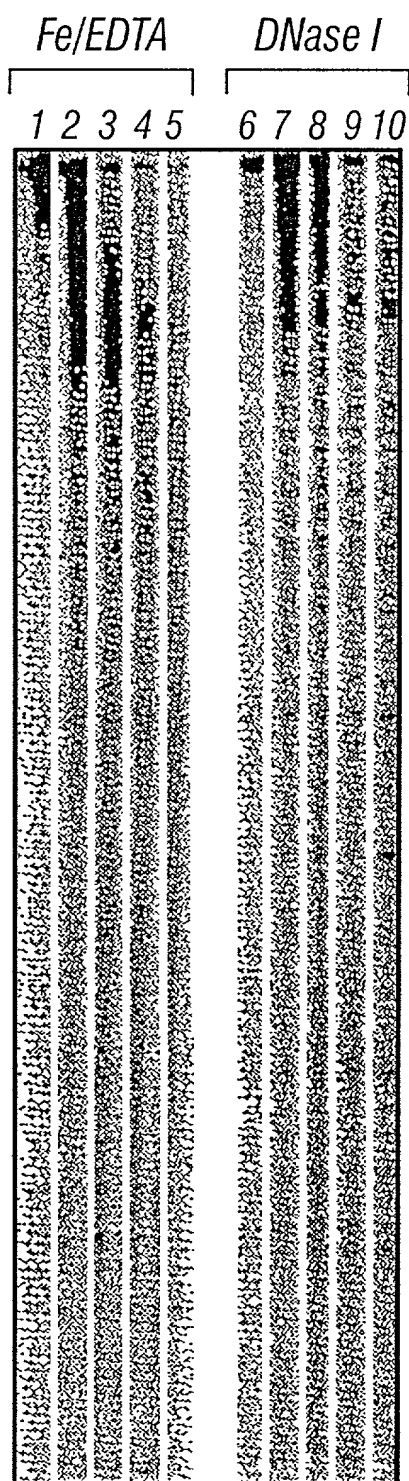


FIG. 27

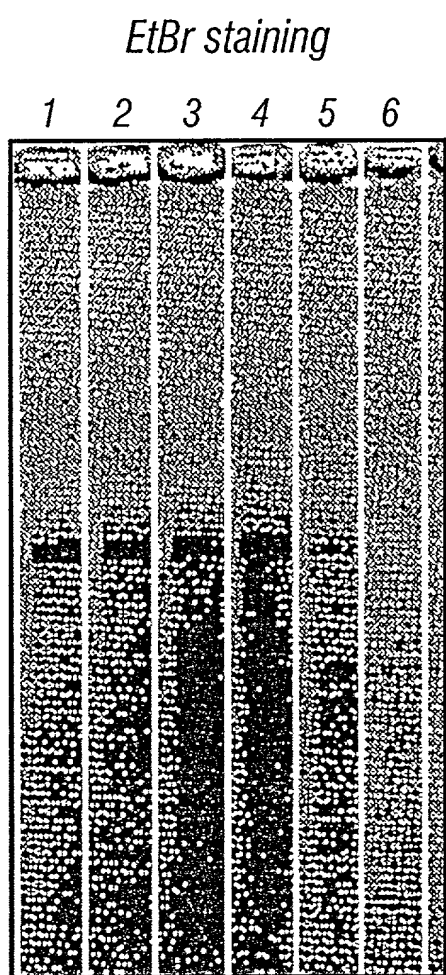


FIG. 28A

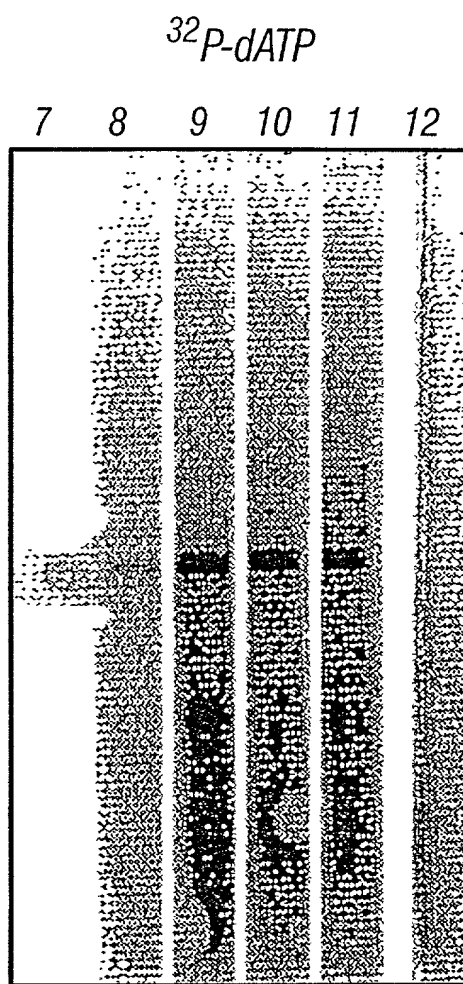


FIG. 28B

FO9080" SHEF0860

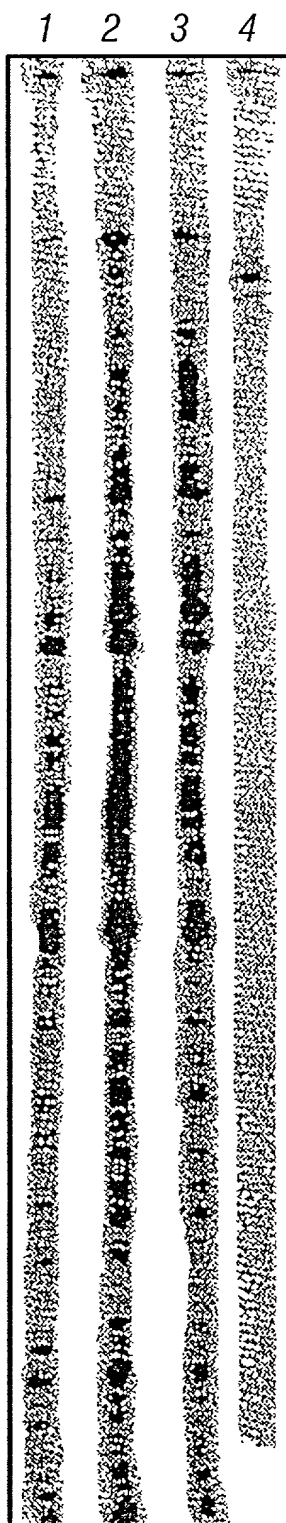
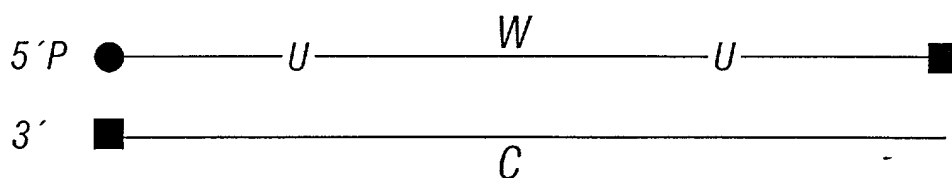


FIG. 29



● - 5' -PHOSPHATE

■ - 3' DIDEOXYNUCLEOTIDE OR NH_3 GROUP

FIG. 30A

| | | |
|----------|-----|-----------------------|
| 5' ————— | X | 3' OH 4 C-X OLIGOS |
| 5' ————— | X | 3' OH 16 C-XY OLIGOS |
| 5' ————— | XYZ | 3' OH 64 C-XYZ OLIGOS |

X, Y AND Z ARE A, T, G OR C

FIG. 30B

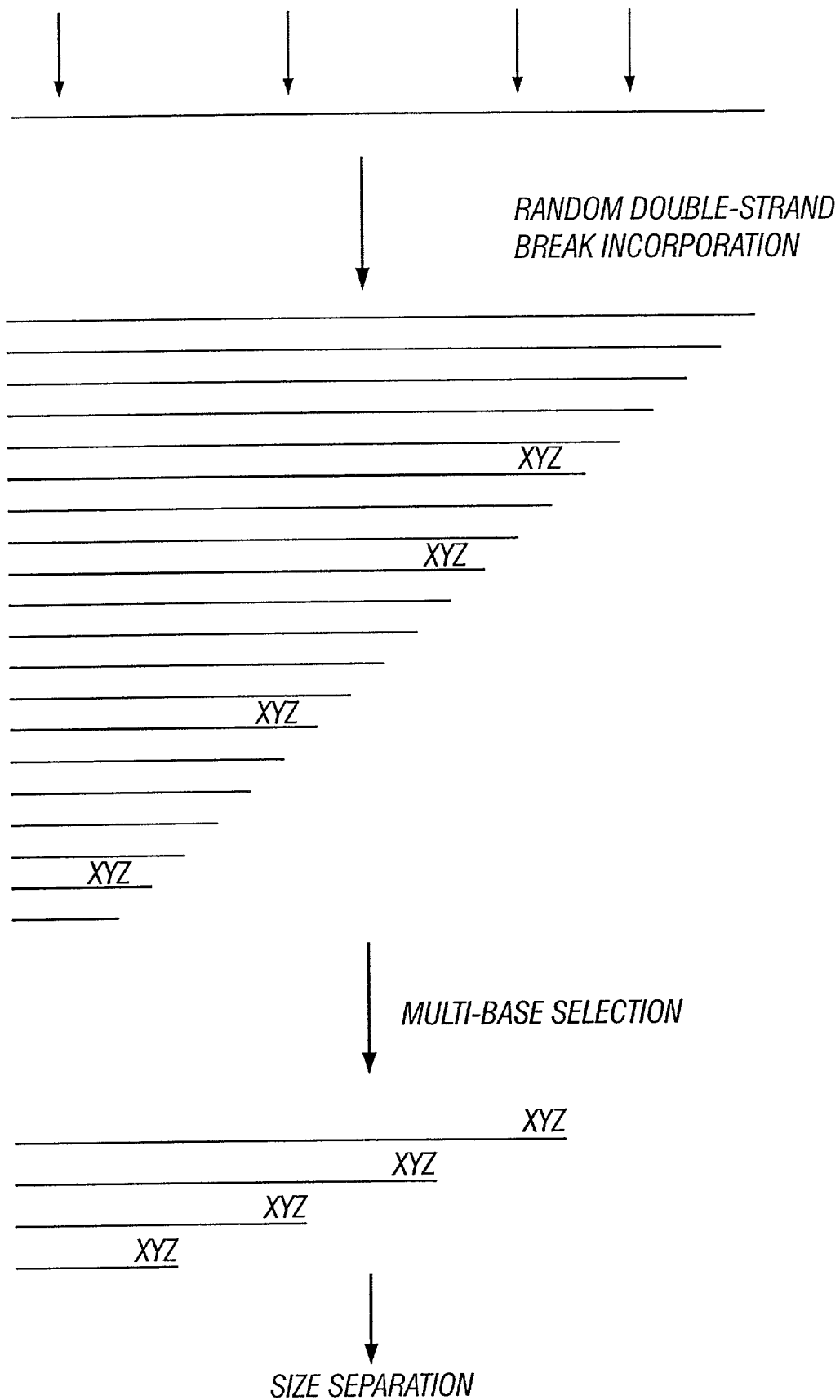


FIG. 31

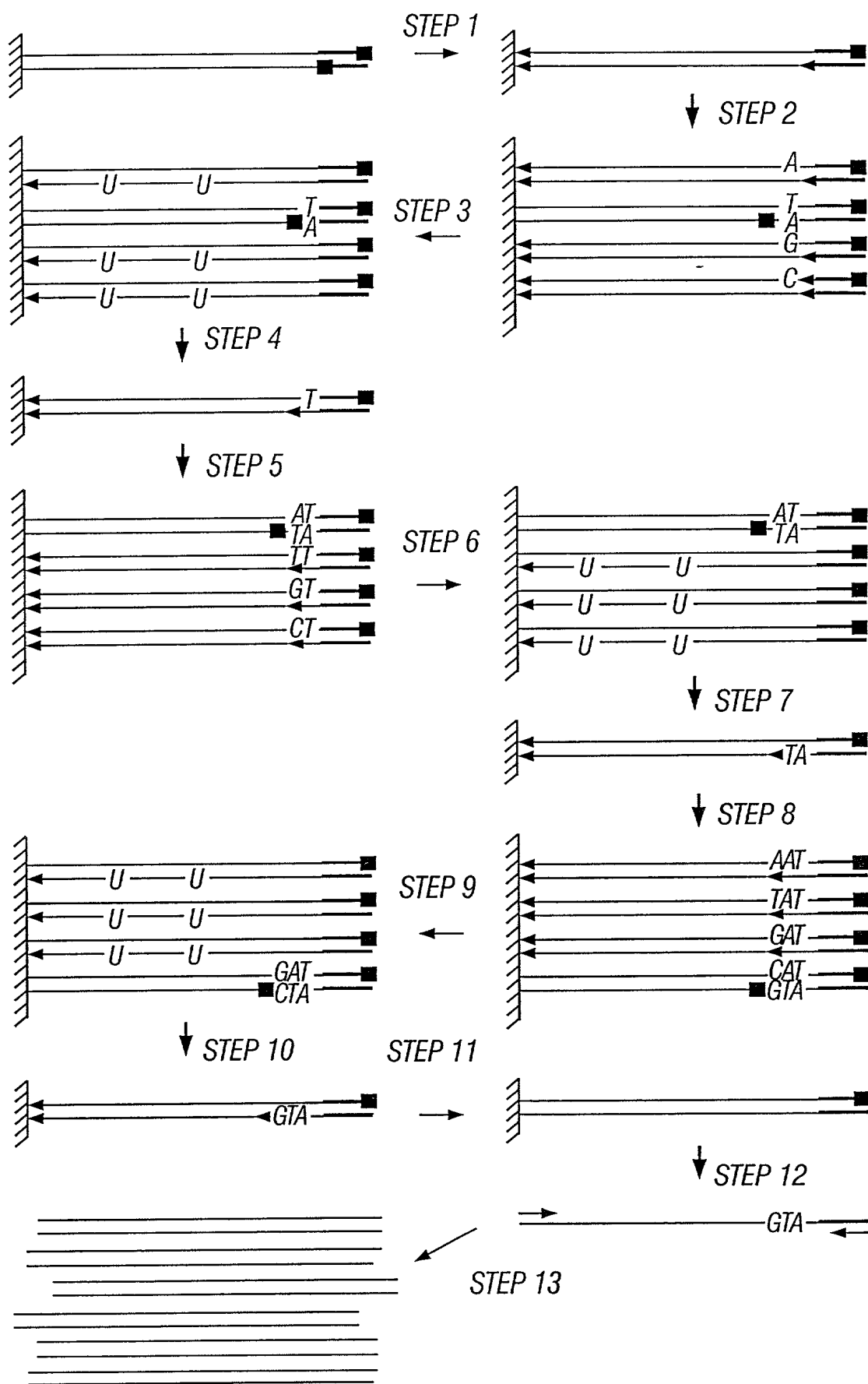


FIG. 32

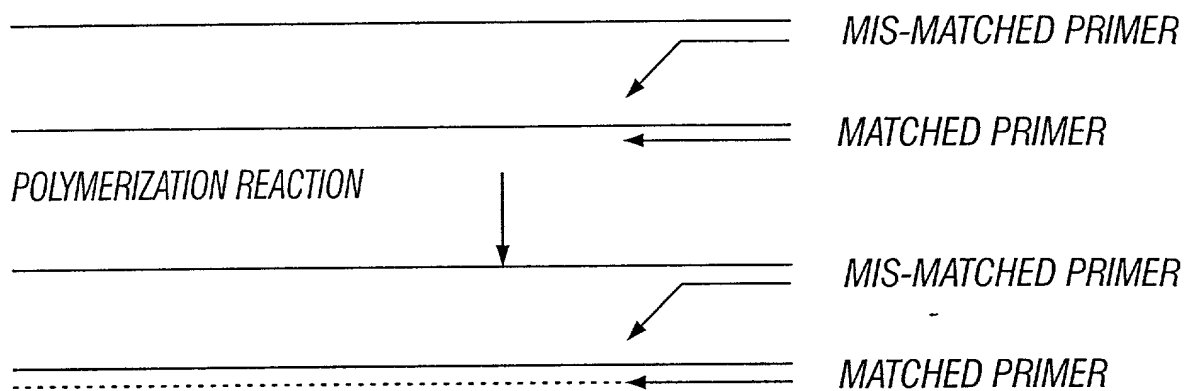


FIG. 33A

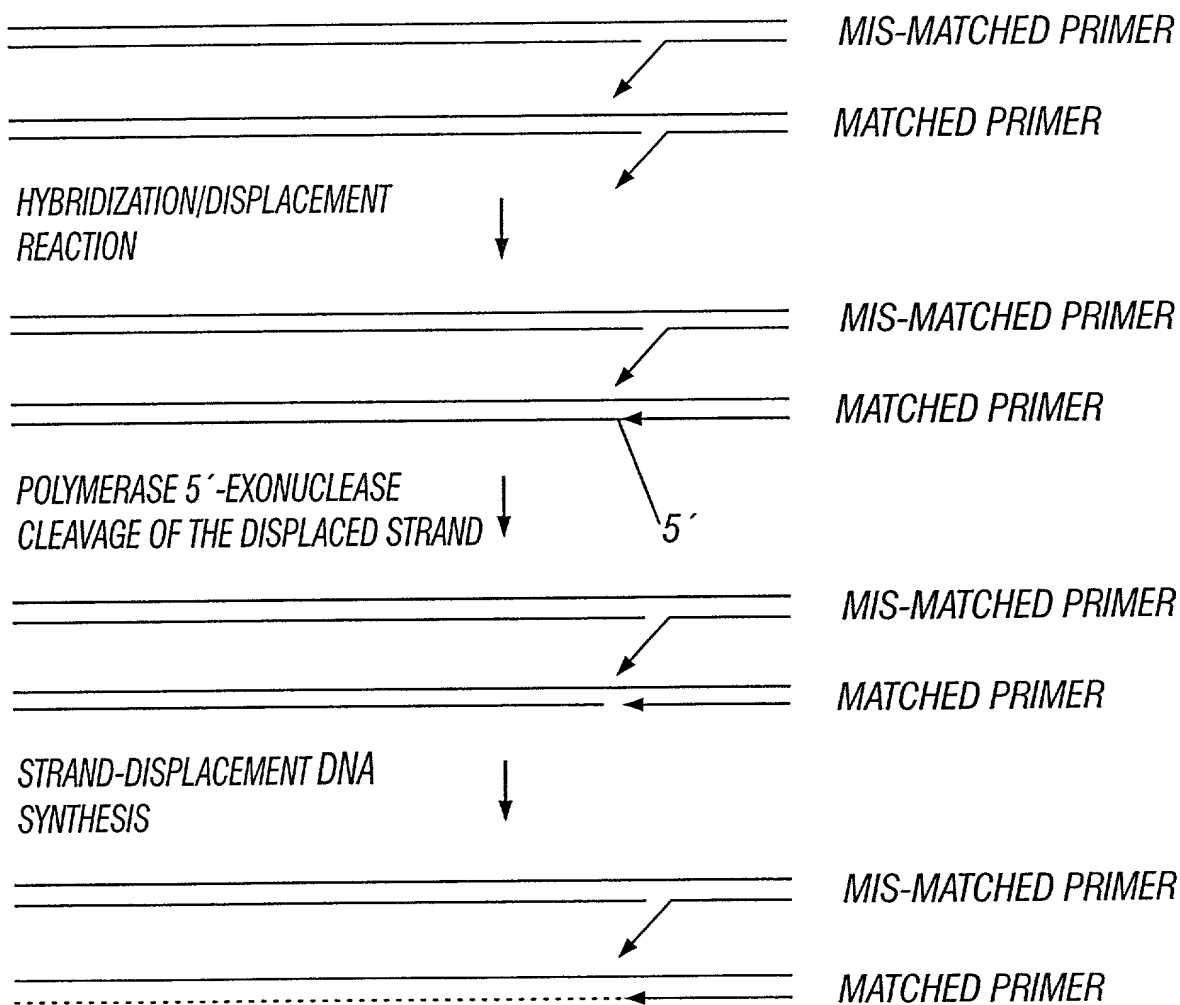


FIG. 33B